

Physiology and ecology of three amphipod species in U.K. estuaries



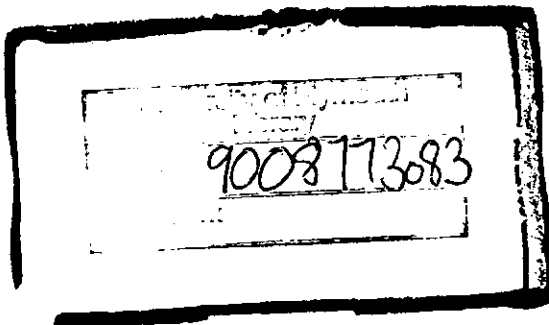
Colette Suzanne Mesher

School of Marine Sciences and Engineering

University of Plymouth

A thesis submitted to the University of Plymouth in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

November 2009



90 0877308 3



Reference Only

LIBRARY STORE

This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognize that its copyright rests with its author and that no quotation from the thesis and no information derived from it may be published without the author's prior consent.

Abstract

Physiology and ecology of three amphipod species in U.K. estuaries

Colette Suzanne Mesher

The physiology and ecology of three amphipod species, *Corophium volutator*, *Gammarus marinus* and *G. chevreuxi* were examined. The physiology of the amphipods were investigated in two ways. The physiological capacity was measured as the rate of oxygen consumption (MO_2 - μ l of oxygen per mg of wet mass per hour), over two time durations, 4 hours (short term) and 5 days (medium term) in a variety of salinities with individuals of all size classes. Overall, larger individuals of all species exhibited lower MO_2 by mass. More specifically, *C. volutator* and *G. chevreuxi* exhibited lowest rates of respiration at salinities between 15 and 20ppt. *G. marinus* was found to have high mortality at 2 ppt, with high rates of respiration measured at 5 ppt. The second set of physiology experiments examined the effect of acclimation temperature, salinity and seasonality on the temperature limits of the three amphipod species. The critical thermal maximum (CTMax) and minimum (CTMin) were investigated using multiple endpoints for summer 2007 and winter 2008 using two test acclimation temperatures (10 and 20°C) and three test salinities (2, 20 and 35ppt). *C. volutator* had the highest CTMax, whereas *G. marinus* had the highest tolerance to cold temperatures with the largest temperature range. The CTMin for male *G. chevreuxi* was found to be significantly lower than females. The ecology of the three amphipod species was studied over a two year sampling period (June 2006 to June 2008) across 11 sites in three estuaries (Avon, Taw/Torridge and Plym). A large amount of variation in the densities was noted with the sex ratios for all species found to have a significant bias towards females at all sites. The density of *G. chevreuxi* fluctuated across site and date equally, compared to *C. volutator* which showed a strong spatial component and *G. marinus* which showed a strong temporal component. A model was developed to incorporate the results of the physiology and ecology investigations. A Binary Logistic Regression approach was adapted and occupancy was found to be a strong predictor of presence/absence data

for all three species. The implications of this model are discussed in relation to a wider physiological and ecological context.

Contents

Abstract	v
Acknowledgements	xix
Author's declaration	xxi
1 Introduction	1
1.1 Ecological background	1
1.2 Estuaries as model systems	11
1.3 Aim and Objectives	12
1.4 Study site and organisms	14
2 The effect of biotic and abiotic factors on physiological capacity	19
2.1 Introduction	19
2.2 Materials and Methods	23
2.2.1 Statistical Analysis	27
2.3 Results	29
2.3.1 <i>C. volutator</i>	30
2.3.2 <i>G. marinus</i>	33
2.3.3 <i>G. chevreuxi</i>	35
2.4 Discussion	38
2.4.1 <i>C. volutator</i>	38
2.4.2 <i>G. marinus</i>	40
2.4.3 <i>G. chevreuxi</i>	40

2.4.4	Conclusions	41
3	Effects of biotic and abiotic factors on physiological tolerances	43
3.1	Introduction	43
3.1.1	Methodology	47
3.2	Materials and Methods	49
3.2.1	Statistical Analysis	52
3.3	Results	54
3.3.1	CTMax	55
3.3.2	CTMin	68
3.3.3	Temperature range	83
3.4	Discussion	90
3.4.1	<i>C. volutator</i>	91
3.4.2	<i>G. marinus</i>	93
3.4.3	<i>G. chevreuxi</i>	94
3.4.4	Conclusions	98
4	Study of the abundance and distribution of the three amphipod species	101
4.1	Introduction	101
4.2	Material and Methods	104
4.2.1	Site and sampling design	104
4.2.2	Statistical analysis	109
4.3	Results	111
4.4	Discussion	128

5 Abundance and occurrence in estuarine amphipods: A conceptual model 137

5.1 Introduction 137

5.2 Material and Methods 142

5.2.1 Model factors 142

5.2.2 Binary Logistic Regression 146

5.2.3 Single species models 149

5.3 Results 150

5.3.1 Whole dataset models 150

5.3.2 Single species models 153

5.4 Discussion 162

6 Final Discussion 165

A Appendix A 173

A.1 Critical thermal maximum 174

A.2 Critical thermal minimum 179

References 185

List of Figures

2.1	Experimental set-up	26
2.2	Mean MO ₂ for <i>C. volutator</i> , short term	32
2.3	Mean MO ₂ for <i>C. volutator</i> , medium term	32
2.4	Mean MO ₂ for <i>G. marinus</i> , short term	34
2.5	Mean MO ₂ for <i>G. marinus</i> , medium term	34
2.6	Mean MO ₂ for <i>G. chevreuxi</i> , short term	37
2.7	Mean MO ₂ for <i>G. chevreuxi</i> , medium term	37
3.1	Experimental set-up	51
3.2	Average CTMax for <i>C. volutator</i> at endpoint of ‘no movement’	58
3.3	CTMax for <i>C. volutator</i> at endpoint of ‘stop swimming’	59
3.4	Average CTMax for <i>G. marinus</i> at endpoint of ‘no movement’	62
3.5	CTMax for <i>G. marinus</i> at endpoint of ‘stop swimming’	63
3.6	Average CTMax for <i>G. chevreuxi</i> at endpoint of ‘no movement’	66
3.7	CTMax for <i>G. chevreuxi</i> at endpoint of ‘stop swimming’	67
3.8	Average CTMin for <i>C. volutator</i> at endpoint of ‘no movement’	71
3.9	CTMin for <i>C. volutator</i> at endpoint of ‘no movement’	72
3.10	Average CTMin for <i>G. marinus</i> at endpoint of ‘no movement’	75
3.11	CTMin for <i>G. marinus</i> at endpoint of ‘no movement’	76
3.12	CTMin for <i>G. marinus</i> at endpoint of ‘stop moving’ for seasons only .	77
3.13	Average CTMin for <i>G. chevreuxi</i> at endpoint of ‘no movement’	80
3.14	CTMin for <i>G. chevreuxi</i> at endpoint of ‘no movement’	81

3.15	CTMin for <i>G. chevreuxi</i> at endpoint of 'no movement'	82
3.16	CTMin for <i>G. chevreuxi</i> at endpoint of 'no movement' for males and females	83
3.17	<i>C. volutator</i> summer trendlines	84
3.18	<i>C. volutator</i> winter trendlines	85
3.19	<i>G. marinus</i> summer trendlines	86
3.20	<i>G. marinus</i> winter trendlines	87
3.21	<i>G. chevreuxi</i> summer trendlines	88
3.22	<i>G. chevreuxi</i> winter trendlines	89
4.1	Example of slate tile	106
4.2	Plankton splitter	107
4.3	Gnathopod 2, palm, of adult male and female <i>Gammarus</i>	109
4.4	Mean log density of <i>C. volutator</i> at different sites	114
4.5	Mean log density of <i>C. volutator</i> at different dates	115
4.6	Mean proportional percentage abundance of <i>C. volutator</i> at different sites	116
4.7	Mean proportional percentage abundance of <i>C. volutator</i> at different dates	117
4.8	Mean log density of <i>G. marinus</i> at different sites	119
4.9	Mean log density of <i>G. marinus</i> at different dates	120
4.10	Mean proportional percentage abundance of <i>G. marinus</i> at different sites	121
4.11	Mean proportional percentage abundance of <i>G. marinus</i> at different dates	122
4.12	Mean log density of <i>G. chevreuxi</i> at different sites	124
4.13	Mean log density of <i>G. chevreuxi</i> at different dates	125
4.14	Mean proportional percentage abundance of <i>G. chevreuxi</i> at different sites	126

4.15	Mean proportional percentage abundance of <i>G. chevreuxi</i> at different dates	127
5.1	Possible factors affecting the abundance-occupancy relationship. . . .	141
5.2	Flow chart for the model process, for both the overall dataset and the individual species models.	146
5.3	Pictographic representation of the binary logistic regression.	147
5.4	Pictographic representation of the expanded statistical model.	148

List of Tables

2.1	Results of the ANCOVA analysis for all three species	29
2.2	Results of ANCOVA analysis for <i>C. volutator</i>	30
2.3	Results of the ANCOVA analysis for <i>C. volutator</i> including durations	31
2.4	Results of the ANCOVA analysis for <i>G. marinus</i>	33
2.5	Results of the ANCOVA analysis for <i>G. marinus</i> including durations .	35
2.6	Results of the ANCOVA analysis for <i>G. chevreuxi</i>	36
2.7	Results of the ANCOVA analysis for <i>G. chevreuxi</i> including durations	38
3.1	Results of the GLM for CTMax	56
3.2	Results of the ANOVA for <i>C. volutator</i> CTMax	57
3.3	Results of the ANOVA for <i>C. volutator</i> CTMax including life stage .	57
3.4	Results of the ANOVA for <i>G. marinus</i> CTMax	60
3.5	Results of the ANOVA for <i>G. marinus</i> CTMax including life stage . .	61
3.6	Results of the ANOVA for <i>G. chevreuxi</i> CTMax	64
3.7	Results of the ANOVA for <i>G. chevreuxi</i> CTMax including life stage .	65
3.8	Results of the ANOVA GLM for CTMin	69
3.9	Results of the ANOVA for <i>C. volutator</i> CTMin	70
3.10	Results of the ANOVA for <i>C. volutator</i> CTMin including life stage . .	70
3.11	Results of the ANOVA for <i>G. marinus</i> CTMin	73
3.12	Results of the ANOVA for <i>G. marinus</i> CTMin including life stage . .	74
3.13	Results of the ANOVA for <i>G. chevreuxi</i> CTMin	78
3.14	Results of the ANOVA for <i>G. chevreuxi</i> CTMin including life stages .	79

4.1	Location of sampling sites	110
4.2	Results of the ANOVA of <i>C. volutator</i>	113
4.3	Results of the ANOVA of sex ratios of <i>C. volutator</i>	113
4.4	Results of the ANOVA of juvenile to adult ratios of <i>C. volutator</i>	114
4.5	Results of the ANOVA of <i>G. marinus</i>	118
4.6	Results of the ANOVA of sex ratios of <i>G. marinus</i>	118
4.7	Results of the ANOVA of juvenile to adult ratios of <i>G. marinus</i>	119
4.8	Results of the ANOVA of <i>G. chevreuxi</i>	123
4.9	Results of the ANOVA of sex ratios of <i>G. chevreuxi</i>	124
4.10	Results of the ANOVA of juvenile to adult ratios of <i>G. chevreuxi</i>	124
4.11	Summary results table	128
5.1	Rankings of each species for physiological and site preferences	145
5.2	Model permutations for the BLR	148
5.3	Results of the basic BLR including all species	150
5.4	Results of the validation for the basic BLR model including all species	151
5.5	Results of the validation for the expanded BLR model including all species	151
5.6	Summary table of all models including all species	152
5.7	Results of the basic BLR for <i>C. volutator</i>	153
5.8	Results of the sediment grain size analysis	153
5.9	Results of the validation for the basic BLR model for <i>C. volutator</i> . . .	154
5.10	Results of the validation for the expanded BLR model for <i>C. volutator</i>	154
5.11	Summary table of all models for <i>C. volutator</i>	155
5.12	Results of the basic BLR model for <i>G. marinus</i>	156
5.13	Results of the validation for the basic BLR model for <i>G. marinus</i> . . .	156
5.14	Results of the validation for the expanded BLR model for <i>G. marinus</i>	157

5.15	Summary table of all models for <i>G. marinus</i>	158
5.16	Results of the basic and expanded BLR models for <i>G. chevreuxi</i> . . .	159
5.17	Results of the validation for the basic BLR model for <i>G. chevreuxi</i> . .	159
5.18	Results of the validation for the expanded BLR model for <i>G. chevreuxi</i>	160
5.19	Summary table of all models for <i>G. chevreuxi</i>	161
A.1	Results of the GLM for CTMax	175
A.2	Results of the ANOVA for <i>C. volutator</i> CTMax	176
A.3	Results of the ANOVA for <i>C. volutator</i> CTMax including life stage .	176
A.4	Results of the ANOVA for <i>G. marinus</i> CTMax	177
A.5	Results of the ANOVA for <i>G. marinus</i> CTMax including life stage . .	177
A.6	Results of the ANOVA for <i>G. chevreuxi</i> CTMax	178
A.7	Results of the ANOVA for <i>G. chevreuxi</i> CTMax including life stage .	178
A.8	Results of the ANOVA GLM for CTMin	180
A.9	Results of the ANOVA for <i>C. volutator</i> CTMin	181
A.10	Results of the ANOVA for <i>C. volutator</i> CTMin including life stage . .	181
A.11	Results of the ANOVA for <i>G. marinus</i> CTMin	182
A.12	Results of the ANOVA for <i>G. marinus</i> CTMin including life stage . .	182
A.13	Results of the ANOVA for <i>G. chevreuxi</i> CTMin	183
A.14	Results of the ANOVA for <i>G. chevreuxi</i> CTMin including life stages .	183

Acknowledgements

This thesis is dedicated to my parents, who supported all my choices, despite not understanding why.

For Julie, who's brilliant idea made marine biology possible.

For Deborah, who knows how hard it is but told me to do it any way.

For Robbie, for always being there.

And finally, for Elias, who can do any thing he wants, even wear that floppy hat again.

This work would not have been possible without a huge amount of help both in the lab and the field. Thanks to Ann Torr, Richard Ticehurst, Alex Fraser, Roger Haslam and Pete Smithers. Thanks for all the help I received from the 6th floor, especially Jaime Davies and Piero Calosi. Lastly, thanks to Penne Donohue, Adam Bates and Hannah Marchant. You were the first and are always the last.

I would like to thank my supervisors Andy Foggo and John Spicer. You took me on blind sight and helped me through all of it.

Finally, I need to thank Richard Barrett. Without you, I would never have been able to answer the question.

Authors declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

Relevant scientific seminars and conferences were regularly attended at which work was often presented.

Signed: 

Date: 8 Jan 2010

Posters and conference presentations :

2009:

United Kingdom Polar Network (UKPN) *Plymouth* Poster presentation: **Understanding physiological tolerance through thermal limits of three amphipod species**

Benthic Ecology Meeting *Corpus Christi, Texas, U.S.A.* Oral presentation: **Location, location, location: What can physiology and ecology tell us about amphipod distribution?**

Society of Experimental Biology (SEB) *Glasgow* Oral Presentation: **Understanding physiological tolerance through thermal limits of three amphipod species**

2008:

Marine Biological Association Postgraduate conference *Aberystwyth* Oral presentation: **Metabolic oxygen of three amphipod species .**

1st International Macrophysiology Workshop *Dartington Hall* Member of organisation committee

2007:

Society of Experimental Biology (SEB) *Glasgow* Poster presentation: **Effects of length of exposure to salinity change in *C. volutator*: Size dependent acclimation?**

Marine Institute Conference *Plymouth* Oral Presentation: **Understanding physiological tolerance through thermal limits of three amphipod species**

2006:

Marine Genomics Europe 2006: Analysing Biodiversity & Life History Strategies course *Kristineberg Marine Station, Sweden* Oral Presentation: **Sex determination in *Ciona intestinalis***

Word count for the main body of this thesis : 27 470

Chapter 1

Introduction

‘Ecology is the total relations of the animal to both
its organic and its inorganic environment’

Ernst Haeckel 1904

1.1 Ecological background

It is necessary to understand the background of any research being undertaken, in order to fully contextualise data gathered. To this end, it is important to review the ecological literature so that when data are analysed it is possible to determine what mechanisms are driving the observed patterns. The broad definition of ecology by Haeckel (1904) at the start of this chapter is considered to be the first attempted, yet it remains as relevant today as at the turn of the 20th century. From these beginnings, a considerable amount of research has been conducted to define and unravel the concepts which comprise current ecology. Charles Elton’s innovative 1927 book ‘Animal Ecology’ described ecology as ‘scientific’ natural history and employed the term ‘niche’ to describe the trophic roles of organisms in their habitats (Elton 1927). This book enabled Andrewartha & Birch (1954) to describe ecology as having two components; the first encompassed older disciplines such as physiology, behaviour, evolution, the second is the study of animals in their ‘habitat’ or ‘niche’ (Elton 1927). An expansion of this def-

1.1. ECOLOGICAL BACKGROUND

inition is found in Andrewartha (1970), where population ecology is described as the study of an organism's abundance and distribution. Krebs (1978) was unsatisfied with Andrewartha's original definition of ecology due to its lack of emphasis on relationships. Krebs therefore defined ecology as 'the study of interactions that determine the distribution and abundance of organisms' (Krebs 1978). Andrewartha & Birch (1984) further developed their definition of ecology to include the distribution and abundance of a species as predicted through study of the environment. Despite several new editions (Krebs 1985, 1994), Krebs' definition remains the same, and is perhaps the most frequently encountered today.

Brown & Maurer (1989) found these definitions of ecology to be microscopic, taking a very narrow view of species in space and time. They proposed focusing attention instead on larger spatial and temporal scales to elucidate patterns in nature which can provide a new way of formulating predictions about the future distribution of all species in a community or geographic area. The developing field of macroecology emerged from this perspective, and represents a top-down, rather than bottom-up approach which helps us to focus in on some of the most relevant questions in ecology (Brown & Maurer 1989, Brown et al. 2004, Gaston & Blackburn 2000). This is not as easy using a reductionist approach. Macroecology represents the quest for ecological interpretation of repeated, persistent, large scale spatial or temporal patterns in the distribution, abundance, morphology, physiology or behaviour of organisms (Chown & Gaston 1999, McGill 2003, Osovitz & Hofmann 2007). It holds as a central tenet the notion that by taking a sufficiently distant view of ecological systems, idiosyncratic 'noise' will disappear, leaving only important generalities (Brown 1995). This approach is complementary, not antithetical, to the traditional ecological approach

(Gaston & Blackburn 2000), and can even be seen to have its roots firmly embedded in the kind of 'ecological web' proposed by Andrewartha and Birch (1954) as well as the biogeographic research of MacArthur (MacArthur & Wilson 1967, MacArthur et al. 1972). By viewing species' autoecology as the dominant driver of distribution and abundance, macroecology links a species to its environment in a more parsimonious fashion than is possible using explicitly relationship-oriented explanations. At the centre of macroecological application of physiological studies lie several generalities, which have been derived from synthetical studies of groups of organisms. These include metabolic scaling laws (West et al. 2002, 2003) which can predict patterns of body size and mass in organisms based upon temperature dependent relative rates of energy usage, and efficiencies of conversion of energy into reproduction (Brown et al. 2003).

One rich area of recent research has been the integration of aspects of autoecology derived from organismal physiology over large temporal and spatial scales into macroecological theory. Chown et al. (2004) states that "macrophysiology" can be defined as: 'the investigation in physiological traits over large geographical and temporal scales and the ecological implications of this variation'. Chown et al. (2004) attribute the beginnings of macrophysiology to several researchers, the earliest being Scholander et al. (1953), whose work concerned large scale studies comparing Arctic and tropical poikilotherms, which was further extended by Lawton (1991). This 'macrophysiology' has been developed greatly since then by authors such as Gillooly et al. (2002, 2003), West et al. (2002, 2003), West & Brown (2004), Ernest et al. (2003) and Brown et al. (2004). More specifically, macrophysiology is considered to deal with three types of patterns; intraspecific, interspecific and assemblage (Gaston et al. 2008, 2009, and references

1.1. ECOLOGICAL BACKGROUND

therein). However, the importance of organismal physiology is not lost, interspecific and assemblage patterns are often derived from means or median data from individuals in a population (Gaston et al. 2009).

Physiology has changed drastically and can now be split into many separate sub-disciplines, forcing a re-definition of terms to incorporate new technologies (Weibel 1997). Further to this, the importance of environmental change in the form of climate challenge has come to the forefront as a critical issue for both macroecology (Gaston & Blackburn 1999) and macrophysiology (Stillman 2003). The research concerning the effects of climate change using macrophysiology has been wide-ranging. Research has taken a variety of forms; testing the implication of a changing environment on animals which currently live at their temperature limits (e.g. Bernardo & Spotila 2006, Stillman 2003, Clarke 1991), to comparison studies of animal populations at different latitudes (e.g. Calosi et al. 2008, Addo-Bediako et al. 2000), comparative phylogenetic studies (e.g. Garland et al. 2005) to physiological variation in insects (e.g. Chown et al. 2007, Terblanche et al. 2006, Terblanche & Chown 2006, Jørgensen et al. 2006), analysis of metabolic biochemistry of thermal tolerance (e.g. Pörtner et al. 2005, Pörtner 2002, Pörtner & Knust 2007) and a great deal more (for a review see Gaston et al. 2009).

It is important to note that the exact definition of macrophysiology is still hotly debated (Chown & Gaston 1999). The question is, at what level do we move from organismal or population physiology to a more general and large-scale macrophysiology? For example, Stillman & Somero (2000) worked on 20 different species of porcelain crabs of the genus *Petrolisthes* which occur on shores from Mexico to Oregon and Chile and Panama. They found that despite being in the same clades, the species have distinctly different critical thermal maxima (CTMax) as a response to their micro-habitats. The

1.1. ECOLOGICAL BACKGROUND

high intertidal species were found to be living at their physiological limits and are therefore at a greater risk due to increasing global temperatures. In contrast, Fangue et al. (2006, 2009) worked on two subspecies of killifish, *Fundulus heteroclitus* (Linnaeus), one northern population from New Hampshire and one southern population from Georgia, U.S.A.. They compared the whole organism metabolic rate with mitochondrial respiration rates. The critical thermal minimum (CTMin) was greater in the northern population, whereas the CTMax was highest in the southern population (Fangue et al. 2006); furthermore the mitochondrial response was found to not necessarily be equivalent to the whole organism response (Fangue et al. 2009). From these examples, it can be seen that the research conducted over a wider geographic range did not necessarily reveal more generalised conclusions. Is physiological work on one species representative of an entire genus or group of animals? Amphipods in the genus *Gammarus* from North American and European species have been shown to be genetically similar despite living in extremely varied environments (Hou et al. 2007). Is the variability found among closely-related species important in the generalised physiological tolerances relating to large-scale geographic distribution patterns of amphipods?

Viewing assemblages or populations of species on a large scale can also be counter-productive; the search for generalities can often lead to real patterns being missed or hidden. The lack of environmental homogeneity in a habitat or amongst species in an assemblage can be masked by scaling across species, communities or geographic scales (Bakker & Kelt 2000). If a set of species found in an environment conforms to a classic $\frac{3}{4}$ scaling rule between body mass and metabolic rate (Gillooly et al. 2001), but individuals within the same species show no such relationship (Clarke 2006), can this

1.1. ECOLOGICAL BACKGROUND

rule really be viewed as an emergent property of organismal physiology and ecology? For example, using abundance data based not on species but on genera can often mask differences in biological traits such as dispersal mode (Statzner et al. 2008). Other methodological errors in execution of studies emerge which further confuse the situation; for example Rakusa-Suszczewski & Lach (1991) measured osmoregulation in individual amphipods without recording their masses, making it impossible to compare their results to any other study. In essence, indiscriminate scaling across life stages or species is inappropriate as, for example, the physiological tolerance of a juvenile cannot be assumed to be equivalent to that of an adult (e.g. Morritt & Spicer 1996a,c), nor the metabolic scaling within species to be comparable to that across species and *vice versa*. The macroecological and macrophysiological approaches must, therefore, be used cautiously as the latest tools researchers can apply to unraveling the factors underlying the distribution and abundance of species, the core of the definition of ecology provided by Krebs.

One macroecological pattern that has been apparent throughout the development of ecology is the positive relationship between abundance and distribution (Darwin 1859, Gaston et al. 2000). This relationship states that a species which is widespread will be locally abundant compared to a species with a restricted distribution which will be locally rare (Gaston et al. 2000). Many theories have been proposed to explain this positive relationship (Hanski 1991, Hanski et al. 1993, Gaston et al. 2000, Freckleton et al. 2005). Gaston et al. (1997) proposed eight different mechanisms to explain the positive relationship between abundance and occupancy. Firstly, it is possible that a spurious relationship can be produced through sampling artefact; a species which occurs at low densities has a lower probability of being sampled than a more abundant

1.1. ECOLOGICAL BACKGROUND

one even if they have an equal extent of distribution (Brown 1984). Evidence to support the artefacts hypothesis is scarce however (Blackburn & Gaston 1998, Blackburn 2004), and despite a debate having raged over the inclusion of effects of aggregation or random placement of individuals that could possibly cause artefactual patterns (Gaston, Blackburn & Lawton 1998, Hartley 1998, Falster et al. 2001), the issue of wholly artefactual generation of patterns seems to no longer be valid (Blackburn et al. 2006).

Next, phylogenetic non-independence can occur where organisms in an assemblage have a shared ancestry, effectively creating pseudoreplicated sets of data points comprising sibling taxa (Gaston et al. 1997b, Levitan 2000, Diniz-Filho & Torres 2002, Prinzing et al. 2004, Ozinga et al. 2004). Clusters of such sibling taxa could generate weak relationships between abundance and occupancy with pseudoreplication effectively generating spurious statistical significance. However, little evidence to support this theory; phylogenetically corrected analyses rarely contradict uncorrected counterparts (Cofre et al. 2007, Rundle et al. 2007, Kockemann et al. 2009), although some research does exist (see Huey & Bennett 1987). Range position could also account for the positive relationship in studies with limited geographic extent of coverage. In any given area some species are less locally abundant and are sparsely distributed due to being at the edge of their distribution while other species which are closer to their range centre are locally abundant and widespread in distribution (Gaston et al. 1997b).

Brown (1995) took the reasoning behind the range position hypothesis one step further encompassing aspects of the organisms' niches, creating a 'resource breadth' hypothesis. Here, generalist species have wide environmental tolerances and are subsequently more abundant and widely distributed, whereas specialists have smaller distributions and are less locally abundant (Bolnick et al. 2002, Kotze et al. 2003, Fernandez &

1.1. ECOLOGICAL BACKGROUND

Vrba 2005, Nagelkerken et al. 2006). The resource availability hypothesis meanwhile, proposes that the positive relationship between abundance and distribution can be explained by some species utilising the most widespread resources resulting in them being similarly widespread whilst others whose limiting resources are scarcer are accordingly less abundant and less frequently encountered (Gregory & Gaston 2000). Habitat selection could also explain a positive abundance-distribution relationship: species may be forced to occupy a wider variety of habitats when they are very abundant compared to when they are less so, leading to a more widely dispersed distribution (Jenkins 2005, Olivier & Wotherspoon 2006). When the diet of a species is flexible and resources are scarce a corresponding increase in niche breadth or degree of generalisation can be seen. For example bumblebees (*Bombus terrestris* (Linnaeus)) increased the number of flower species they visited when conspecifics are present (Fontaine et al. 2008).

Perhaps the most persuasive theories explaining the patterns deal with population dynamics. Firstly, metapopulation dynamics may result in positive abundance-range size relationships (Hanski & Gilpin 1991, Hanski & Gyllenberg 1997, Hanski 1998). Metapopulation-based explanations can be broken down into the carrying capacity and the rescue effect hypotheses (Gaston et al. 1997). The carrying capacity of a species is reflected in its local abundance and regional distribution, where all areas are equal and available to that species. Highly abundant species thus, are considered to have high carrying capacities with low extinction rates and/or a high colonization rates but can be limited by resource availability or use. The rescue effect hypothesis meanwhile only considers immigration; a positive relationship can be obtained if immigration is high enough to prevent extinction, the more immigration that occurs the greater the number

1.1. ECOLOGICAL BACKGROUND

of patches that are occupied. Finally, the vital rates hypothesis (Holt et al. 1997, 2004) is similar to the rescue effect in that immigration must be adequate to allow the species to persist in a given habitat, but it differs in that immigration *per se* does not determine the local abundance. The positive relationship instead is generated by the rate at which births exceed deaths differing for different species.

One of the ways to deduce the relevant questions which will determine the cause of the abundance-occupancy relationship is by modeling the abundance and distribution of the species in question. Gaston & Blackburn (1999) pointed out that there was a lack of proper models and rigorous testing in macroecology, which results in a community analysis where no hypotheses can be rejected outright, as it is important to consider all mechanisms. In a review Gaston et al. (1997b) referenced almost a hundred papers which focused on a particular group of animals. Of those, about 30% deal with insects, with almost half of them focusing on butterflies. Another 30% of papers refer to models describing birds, with the remaining papers focusing upon plants and fish, with only a single paper each on barnacles and molluscs. More recently, Blackburn et al. (2006) reiterated essentially the same summary of the state of the field, and again drew attention to the dearth of contributions from marine science. There appears to be a gap in the literature with respect to ecological models pertaining to marine and particularly estuarine organisms. However, there has been a resurgence in ecological modelling appearing in recent research (e.g. Thrush et al. 2008, Hewitt & Thrush 2009). Foggo et al. (2003) studied the abundance and distribution of estuarine macroinvertebrates, whilst Frost et al. (2004) studied abundance and occupancy of macrofauna on sandy beaches. Neither found major difference between the distributions of species with distinctly different reproductive strategies, indicating that the mode of dispersal was not the likely

1.1. ECOLOGICAL BACKGROUND

mechanism creating the positive abundance-occupancy relationship. However, more recent contributions by Foggo et al. (2007) suggest that dispersal does affect the nature of abundance-occupancy relationships in more connected, fully marine systems. Neither set of findings appears conclusive, with the role of habitat and patch connectivity appearing to be important in determining whether biological traits such as reproductive and developmental biology play a part in structuring the relationship between abundance and distribution. Further evidence of the importance of such strategies in determining abundance-occupancy relationships can be found in non-marine oriented work. A wider niche breadth along with an understanding of biological traits and how these can be modified by environmental factors is argued to be a determinant of abundance (Statzner et al. 2008). The difficulty with this was demonstrated by Gaston et al. (2008); they noted a lack of basic information such as body size and animal fecundity for a portion of the world's avian species. This makes formulating generalities regarding abundance and occupancy impossible. Another example exists for fish found in freshwater lakes in south-eastern France (Mason et al. 2008). The abundance of fish species was thought to be determined by niche size based on resource use. Abundant species avoid overlap due to specialised feeding modes and prey species. However, due to lack of data on environmental factors, life stage and resource availability, limits to the conclusions reached in this research exist. However, examples of research with a more comprehensive set of data can be found. Energy limitations due to a large body size of insectivorous birds in the Mediterranean and Temperate forests of Chile are associated with low abundances (Cofre et al. 2007). Habitat specificity was found to limit their latitudinal range and this was further confounded by habitat fragmentation and small clutch sizes (Cofre et al. 2007).

1.2 Estuaries as model systems

Estuaries are unique environments and are ideally suited to test macroecological models. The argument brought forth by Gaston and Blackburn (1999) regarding the lack of proper testing of models arises again in Attrill's 2002 paper on diversity trends in estuaries. Attrill (2002) points out the common estuarine diversity model is based on Remane's work from 1934, a model actually developed for the tideless Baltic Sea, making it inappropriate for use in estuaries. Estuaries are considered to be transitional areas between rivers and seas but have not been properly investigated as such (Attrill & Rundle 2002). Attrill (2002) thought the use of Remane's model to be inappropriate and developed a new model based on a dataset describing the Thames estuary. This model indicates that salinity variation is one of the main factors affecting diversity and distribution of estuarine species, with salinity range experienced being key (Attrill 2002). A large body of work exists which categorises terrestrial and marine systems, yet the unique environment created by the river and sea interface in estuaries is often overlooked. Attrill et al. (2001) showed that the latitudinal diversity cline apparent in many terrestrial environments is also present in estuaries; estuarine diversity increases with ambient environmental temperature, perhaps as a result of tropical communities having had longer to settle a temperate estuary. Attrill & Rundle (2002) continued to categorise estuaries using ideas developed for use in terrestrial ecology. They categorised estuaries as being unusual due to having two transitional zones, ecoclines, rather than just one. The river to the mid-estuary is one ecocline and mid-estuary to sea is the second. Attrill & Rundle (2002) argue that there are no true estuarine species, merely two distinct groups of animals, freshwater and marine, living at the edge of their limits in mid-estuary. This research presents estuaries as unique environments able to

1.3. AIM AND OBJECTIVES

contribute to the abundance-occupancy debate by placing definable boundaries on a species distribution due to its ability or lack thereof to tolerate salinity fluctuations. Estuaries therefore represent challenging environments for organisms to live in, and place demands upon many aspects of their biology. This also makes them interesting and challenging environments in which to test the contributions of physiology to distribution and abundance, and therefore in which to bring together macroecology and physiology.

1.3 Aim and Objectives

Aim The main aim of this work is to integrate physiological and ecological studies of a set of model estuarine organisms to seek inter-relations between physiological capabilities and spatio-temporal patterning of distribution and abundance. Ultimately, the goal is to construct a model with both conceptual and mathematical elements, incorporating physiological and ecological data. The model can then be used as a tool to predict aspects of distribution and abundance of the organisms concerned.

Objectives The model operates on three key parameters, which define the following objectives:

Define physiological tolerance/capacity in key selected species The first step to constructing this model is to determine the physiological capabilities and limits of the species considered. Salinity and temperature tolerances are critical factors affecting the survival and distribution of species in estuaries (eg: Attrill 2002, Attrill & Power 2004). Thus, the first two data chapters of this thesis will investigate the metabolic oxygen utilisation of the species' as a proxy for physiological performance or capacity (Chapter 2) and critical maximal and minimal temperatures as a proxy for physiolog-

1.3. AIM AND OBJECTIVES

ical tolerance (Chapter 3). To avoid confusion, a single unit for salinity will be used throughout this thesis; parts per thousand (ppt).

Determining ecological context Once the physiological limits have been established, it is then possible to place these into context with respect to the ecological data collected. Therefore, it is necessary to quantify actual abundances and distributions of species in space and time. Thus, chapter 4 will document the abundance, distribution and demographics of the species within their respective habitats.

Construct and validate model The penultimate chapter (Chapter 5) discusses the construction and validation of the model using well-established ecological and mathematical approaches based around techniques such as logistic regression. The final chapter is a general discussion, examining the physiological and ecological chapters in a wider context.

Previous researchers have often built models describing abundance and distribution in estuaries on the basis of ecological data alone (see Attrill et al. 2001, Foggo et al. 2003), this could lead to a misrepresentation of the niche size of estuarine organisms as the physiology of the species is not taken into account. It is for this reason that an integrated approach focusing on both physiology and ecology is necessary to gain a more complete understanding of why animals live in a given habitat. The predictive tool will take the form of a model, its purpose being a platform for the prediction of species population density and spatial distribution. The possible applications of such a model are wide-ranging, provided it is flexible enough to deal with a number of simultaneously differing parameters and can predict interactions between the inputs. The proposed model will be designed to indicate predicted population density and spatial distribution given input parameters of salinity, temperature and local proportion

1.4. STUDY SITE AND ORGANISMS

of sites occupied.

The derivation of such a model does have certain limitations, which are broadly outlined here. Predictions can only be made about a species if its abundance and distribution is limited by physiological processes, within a tractable system, with distinct habitat patches. For example, the salinity and temperature in the pelagic environment in the open ocean is relatively stable compared to a benthic habitat in an estuary, where salinity can change several times a day with the incoming and outgoing tides, or the amount of freshwater input (Barnes 1974). The tides can also leave habitats exposed and this can lead to large fluctuations in temperature (Newell 1979). Habitat availability can also be limited by variable sediment types and patchy vegetation. The physiological stress as well as limited habitat availability make estuaries an ideal habitat for development of such a predictive tool. Although estuaries are considered to be very variable environments, many species still choose to withstand these changes in salinity and temperature as they offer shelter and an abundance of nutrients not available elsewhere (Barnes 1974). In order to increase the applicability of the tool, choosing a study organism which is widespread and experimentally tractable is crucial. Amphipods are one such group of organisms.

1.4 Study site and organisms

Amphipods were chosen as the study organisms for this study; they are abundant in a variety of estuaries locally, and are tractable subjects for laboratory studies. They are found throughout the world and are often present in variable environments such as estuaries as many species have wide salinity and temperature tolerances (Sexton 1928, Segerstråle 1959, McLusky 1968, Aikins & Kikuchi 2001). Amphipods are known

1.4. STUDY SITE AND ORGANISMS

to be reliable laboratory animals due to low mortality and simple feeding regimes (Gaston & Spicer 1998). Two abundant and widespread species, *Corophium volutator* (Pallas, 1766) and *Gammarus marinus* (Leach, 1815), as well as a less abundant species *Gammarus chevreuxi* (Sexton, 1913) with a more localised distribution were chosen as study organisms. *Corophium volutator* is a common species of amphipod in estuaries, ranging across the European coasts into the North Atlantic and American coasts (Lincoln 1979). It has an apparent wide salinity tolerance, ranging between 2.6 and 46 ppt, which increases when supplied with a suitable substrate (with a biofilm as a food source) due to increased energy requirements in extremely low salinities (McLusky 1967). *Gammarus marinus* (also known as *Chaetogammarus marinus*, *Marinogammarus marinus* (Lincoln 1979) and *Echinogammarus marinus* (Ford et al. 2003); hereafter referred to solely as *Gammarus marinus* (Bellan-Santini 2009)) is a widespread species which is often used in toxicological studies (Ford et al. 2003, Clason et al. 2004). *Gammarus marinus* is considered to be a true marine species (Sexton & Spooner 1940) but has been shown to tolerate salinities between 0 to 35 ppt with preferences for 28 ppt to full strength seawater (Bettison & Davenport 1976). *Gammarus chevreuxi* meanwhile is a rare species with its worldwide distribution being limited to only a few reported locations in the U.K. (where it appears to be locally abundant - see Lincoln (1979)), France, and an isolated case in Portugal (Crawford 1937, Stock 1967). Yet, there are contradictory reports where *G. chevreuxi* is also considered to be abundant in Europe and North Africa (Sexton 1928, Subida et al. 2005). Lincoln (1979) states that the main distinguishing feature of *G. chevreuxi* is the long curled hairs on large adult males as there are many anatomical similarities to other species of *Gammarus*. This could lead to an under-recording of the species; north Wales is presently the northern extent of its recorded range (Lincoln 1979). Early physiology

1.4. STUDY SITE AND ORGANISMS

experiments on *G. chevreuxi* showed it to be highly tolerant of brackish (25 ppt) water with respiratory rates comparable to *G. marinus* and *G. pulex* (Linnaeus) (Lowenstein 1935). In Portugal, *G. chevreuxi* were found to inhabit low salinities (up to 4 ppt) and were most abundant during the winter months with an average water temperature of 12°C (Subida et al. 2005). *Corophium volutator* is considered to be a true estuarine species, and these physiological tolerances contrasts with the more marine *G. marinus* which in turn is physiologically very different from *G. chevreuxi*. These stark differences in habitat choice and physiological limitations make these amphipod species ideal for comparison in order to elucidate the main factors limiting each species distribution. The ready availability of these species of amphipods and their varying physiological characters, distributions and abundances makes them ideal study organisms for both laboratory and field sampling, and for the purposes of this work.

The Avon, the Plym and the Taw/Torridge estuaries were recognised as ideal test systems. Three sites were selected on the Avon and Plym estuaries and four on the Taw/Torridge estuary system. *Gammarus chevreuxi* and *G. marinus* were collected from the Avon and the Plym estuaries; *C. volutator* from the Avon and the Taw/Torridge. Although the three species of amphipods chosen live in ecologically different niches, they are all found within close proximity at each site in the estuaries studied. Sites where the specific gammarids were collected had flowing water with stony bottoms (*G. chevreuxi*) and the presence of patches of *Fucus* spp. seaweed (*G. marinus*). *Corophium* meanwhile has a patchy distribution on large areas of mud and muddy sand flats, which are exposed at low tide (Meadows 1964a); both the Avon and the Taw/Torridge have well established populations. These systems were chosen after extensive preliminary research on the above estuaries as well as the Tavy, the Exe and

1.4. STUDY SITE AND ORGANISMS

the Tamar. In order to be included in a site, a species had to be found at a minimum density to allow abundance to be estimated meaningfully. The sites chosen reflect different aspects of their localities: the Avon is a southern English estuary which is well maintained with grade A water quality (Buck 1997); the Plym is also a southern estuary but it is much more polluted by the china clay quarrying activities, the city of Plymouth and dockyards (Buck 1997) and by virtue of a considerable volume of heavy boat traffic due primarily to Sutton Harbour (Smith et al. 1995); the Taw/Torridge is a northern site but is well maintained environmentally with the exception of the Appledore boat yard and low levels of commercial and military boat traffic. The selection of these sites was firstly based on the presence of the animals as well as site accessibility and habitat type. Some limits were imposed due to the lack of sufficiently large *Fucus* spp. patches or flowing water on the Avon; the Taw/Torridge estuaries meanwhile were treated as a single system due to accessibility and safety while sampling, and the lack of adequate numbers of discrete sites within each of the two systems.

Chapter 2

The effect of biotic and abiotic factors on physiological capacity

2.1 Introduction

Amphipods are an ubiquitous component of estuaries due in part to their ability to cope with varying salinities; *Corophium volutator*, *Gammarus marinus* and *G. chevreuxi* are common species in estuaries across Europe (McLusky 1968, Bettison & Davenport 1976, Subida et al. 2005). An animal's ability to respond to changes in salinity can be a significant determinant of its distribution (see Attrill 2002). There are numerous ways in which to measure the response of animals to a change in salinity. For example, McLusky (1967) used choice experiments where *C. volutator* were allowed to choose their preferred salinity by swimming or crawling between compartments of different salinity. Previous studies agreed with the findings of this work and showed *C. volutator* to have a wide salinity tolerance, being able to survive from nearly freshwater to hypersaline conditions (47 ppt) (McLusky 1967). Acclimation studies showed that when suitable substrate is available, the distribution of *C. volutator* might be limited by exposure to low salinities; reproduction can only occur when the water is at a minimum salinity of 7.5 ppt (McLusky 1968). However, waiting for reproduction to occur and the subsequent development of the young is very time consuming as a laboratory

2.1. INTRODUCTION

or field-based approach to studying the physiological constraints upon the organisms in their habitat. A more immediate approach is the use of oxygen consumption as a measure of physiological capacity, as a shift in requirements away from metabolic processes (such as reproduction) can occur in order to maintain normal respiration rates (McLusky 1969).

Oxygen consumption data on their own are not very informative, but it is assumed that the animals' oxygen consumption is directly related to their metabolic rate and thus energetic costs (Lofts 1956). This relationship has been tested previously, by measurement of direct heat dissipation during salinity challenge of crabs (*Rhithropanopeus harrisii* (Gould), but this method can be expensive and extremely time consuming (Normant & Gibowicz 2008). As the maintenance of osmotic balance has a high energetic cost (Potts 1954), oxygen consumption is considered to be the preferable method. In this case the animal's mass specific oxygen consumption rate is expressed as rate of O_2 consumed (MO_2). Results from MO_2 experiments can be interpreted in general terms by considering that deviation from baseline oxygen consumption requires energy expenditure, and thus is considered to represent 'reduced capacity'. However, on occasion it is not always possible to obtain a baseline, for example, in a widely fluctuating natural environment. In these cases, results have to be compared to one another to estimate in what conditions the lowest oxygen consumption occurs, with this set of conditions then considered to be those producing 'high capacity' and representing the 'baseline'.

Traditionally, quantitative analysis of organismal oxygen consumption has been based on the widely accepted assumption that a change in energy expenditure results in a direct change in metabolic rate (Schmidt-Nielson 2004). Most recently Brooks & Mills

2.1. INTRODUCTION

(2006) were able to quantify the impact of acclimation of *G. pulex* to different salinities with the use of gill Na, K ATPase activity. The highest rates of enzyme activity were found in gammarids acclimated in the most dilute medium; a significant increase in haemolymph sodium concentration was noted when freshwater acclimated animals were subjected to 12 ppt. A high enzyme activity rate would presumably require more energy, which would have to be diverted from other processes such as growth and reproduction. This research is significant because it uses a direct measurement of energy expenditure to quantify an animal's response to a change in salinity. The research supports the assumption that oxygen consumption can be used as an indication of the metabolic response to environmental stress. Previously, results from experiments using oxygen consumption have been varied. For example, *Dikerogammarus villosus* (Sowinsky) from the River Rhine was seen to have maximum oxygen consumption at 20°C with the minimum at 5°C; animals were able to tolerate salinities up to 20 ppt, with baseline oxygen consumption obtained between 0 and 10 ppt (Bruijs et al. 2001). Results from experiments using *G. oceanicus* (SegerstrAële) meanwhile indicated an increase in oxygen consumption with an increase in temperature, with results affected by the moulting state of the animals (Halcrow & Boyd 1967).

Although tractable techniques for studying physiological capacity exist, there are still many 'unknowns' in the available literature. Previous work has shown developmental differences in physiological capacity in several crustacean species (e.g. Morritt & Spicer 1996a,b, 1999, Spicer & Eriksson 2003, Spicer & Stromberg 2003), however most early research was conducted only on adults. For example, Halcrow & Boyd (1967) measured locomotory activity as a proxy for standard metabolic rate in *G. oceanicus*, but they only used large adult males. The physiological limits of *Mytilus*

2.1. INTRODUCTION

edulis (Linnaeus) meanwhile would not have been found unless larvae were used, as the combination of temperature and salinity limited their growth outside of 25-35 ppt at 15°C (Brenko & Calabrese 1969). In a review of oxygen uptake in gammarids, Sutcliffe (1984) determined that the absolute rate of oxygen uptake was not directly proportional to body mass, and therefore a standard comparison of results across different experiments is not possible if data describing mass are neglected as has often been the case (Spicer & Stromberg 2003).

When these problems are corrected for, it is possible to further our understanding of these complex physiological results by placing them into an ecological context to see whether these data can explain patterns in abundance and distribution. The oxygen consumption of an animal exposed to changes in salinity over various time periods may give insight into how the animals have adapted to salinity changes experienced over daily tidal cycles, e.g. when *C. volutator* is adequately acclimated to a new salinity, the animals do not show any significant changes in oxygen consumption compared to baseline levels (McLusky 1969). This could be related to a diversion of energy requirements (McLusky 1969) as *C. volutator* has been shown to have a reduced growth in very low salinities (McLusky 1967). Indeed, early research on two species of *Palaeomonetes* shrimp concluded that the increase in metabolic rate when exposed to a change in salinity had energy costs, and these went beyond the osmoregulatory mechanism into other physiological processes (Lofts 1956). Hence, the distribution of an animal may be limited not by the maximum and minimum salinities it can survive in, rather by the energetic costs associated with living in a physiologically challenging environment over its entire lifespan.

There are thus a variety of factors which influence oxygen consumption and which

2.2. MATERIALS AND METHODS

may impact upon other metabolic processes. The aim of this chapter is therefore to investigate the effect of biotic and abiotic factors on physiological capacity of the three amphipod species, *C. volutator*, *G. marinus* and *G. chevreuxi*. The biotic factor considered is body mass and the abiotic factors are salinity and acclimation (time). The physiological capacity will be measured as rates of oxygen consumption - MO_2 . In particular, the studies are designed to investigate short term effects of salinity stress on animals to determine their responses to acute shock (4 hours), whilst contrasting medium term (5 days) experiments are designed to determine animals' outright acclimatory capacities. Therefore, the objective of this chapter was to measure the oxygen consumption of these three amphipod species over different lengths of exposure to test for acute and acclimatory responses to changes in salinity.

2.2 Materials and Methods

Corophium volutator were collected from the Avon Estuary (National grid reference SX 666 439). The animals were collected from a small mud flat accessible from the road, hereafter referred to as the 'Tidal Road' site, collected by scraping the top 1cm of muddy sediment and sieving it through a 500 μm sieve bag *in situ*. The isolated animals were then placed in a bucket with sediment to be returned to the laboratory and kept at 20 ppt. Both species of *Gammarus* were collected from the Plym Estuary (National grid reference SX 540 566) at the Chelson Meadow site and returned to the laboratory where they were kept at 35 ppt and 20 ppt respectively. *Gammarus marinus* were collected by hand from under dense patches of *Fucus vesiculosus* (Linnaeus) and *G. chevreuxi* were collected by kicking sediment in the centre of an outflow into a standard FBA net. All animals collected were put into buckets with appropriate habitat,

2.2. MATERIALS AND METHODS

Fucus and rocky sediment, respectively. These salinities were selected to reflect the average experienced over a typical tidal cycle based on preliminary observations. All animals were kept in a 12:12 light:dark cycle at 15°C. *Corophium volutator* and *G. marinus* were kept in aquaria (vol. = 15L) tanks covered with cling film (to reduce evaporation) held in place by plastic tank covers. Three aquaria for each species were maintained with suitable substrate and the water was changed weekly. In order to allow for larger rocky substrate, *G. chevreuxi* was kept in a covered aquarium (vol. = 50L) with a filter. Water changes were also undertaken weekly.

Two sets of experiments were undertaken, one short term (4 hours) and one medium term (5 days). The short term experiment measured the oxygen consumed by the animals after four hours of exposure to a change in salinity. For each salinity, 15 individual animals were used for each species. In the medium term experiment, animals were taken from their laboratory acclimation tanks and exposed to a change in salinity for five days, in individual flow through containers (see fig. 2.1), prior to being transferred for measurement of oxygen consumption using the same protocol employed in the 4h trial. For both experiments, in order to measure the oxygen consumption, individual animals were placed in darkened vials covered with gauze to reduce activity (see Agnew & Taylor 1985, Spicer 1998), and left in aerated aquaria (considered to be normoxia or 100% oxygen) for 30 minutes. After this time, the animals were sealed in their vials and left for four hours. Once the four hour time period was completed, a sample of water (vol. = 10mL) was taken from the vial and oxygen concentrations determined using a Strathkelvin Instruments (Glasgow, Scotland) model 781 closed respirometry meter (see fig. 2.1). Oxygen consumption or metabolic oxygen (MO_2 - μl of oxygen per mg of wet mass per hour) were used. After experimentation all ani-

2.2. MATERIALS AND METHODS

mals were blot dried and weighed on a Fisherbrand PS-100 precision balance (100 g x 0.1 mg) before being preserved in 70% ethanol, in order to be examined individually to determine length and to ensure the correct species in smaller individuals.

The set up of the medium term (5 days) experimental equipment was time consuming as many modifications to the flow-through containers were required in order to ensure consistency and containment of the animals. Preliminary experiments found that it was extremely difficult to keep the more saline-adapted *G. marinus* in the low salinity conditions for extended periods of time due to high mortality. Therefore, it was not possible to expose all species to the same range of salinities. For the short term experiment therefore, *C. volutator* was kept in 20 ppt and the experimental salinities were 2, 5, 10, 15, 20, 25, 30 and 35 ppt, with only 2 ppt not being repeated in the medium term experiments. *G. marinus* was kept in 35 ppt until the start of the experiments and then exposed to 2, 5, 20 and 35 ppt, with the medium term experiments using 5, 20 and 35 ppt. The salinities used in the short term for *G. chevreuxi* were 2, 5, 15, 20 and 35 ppt, with 2 ppt being omitted in the medium term experiments. For each salinity and temperature combination, 15 individuals were used per trial.

2.2. MATERIALS AND METHODS

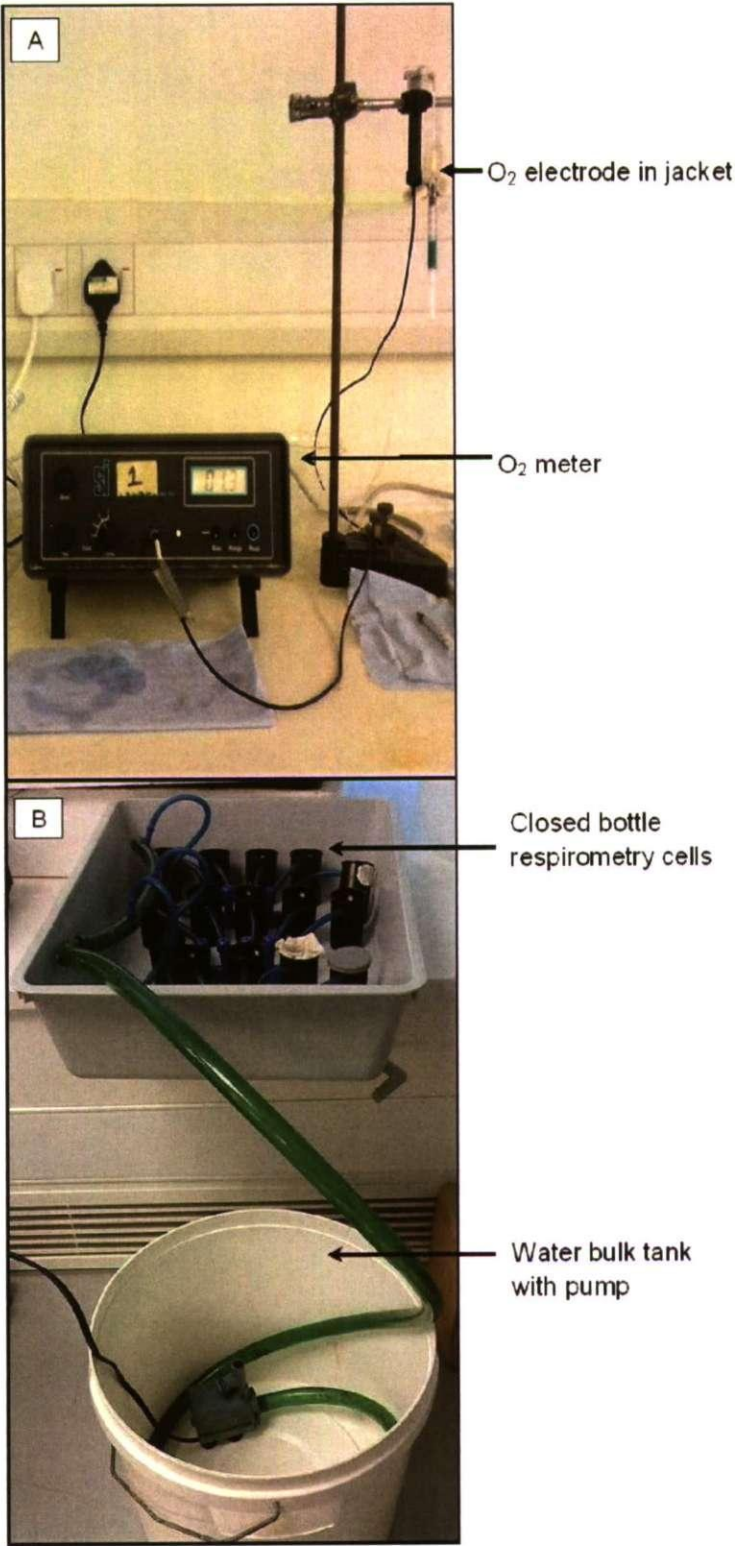


Figure 2.1: Experimental set-up; (a) Closed respirometry system (Picture courtesy of P. Donohue) and (b) 5-day flow-through system.

2.2.1 Statistical Analysis

There was significant variability in available animal size within and between species, and depending on exact timing, within the season. For both these reasons it was not always possible to obtain data across the entire size range for each species in all trial combinations. In order to visualise the data as a whole and make standardised comparisons across sizes of organisms, acclimation, durations and salinities, a regression-based line fitting approach was employed to extrapolate results for typical 'small' and 'large' animals in each species. Standardised sizes were derived from the average minimum and maximum mass data for each species. The 'small' animals for *C. volutator* and *G. chevreuxi* were 2 mg and for *G. marinus* 10 mg. The 'large' mass for *C. volutator* was found to be 10 mg, for *G. chevreuxi* was 13 mg and for *G. marinus* 100 mg. The \ln (wet mass) vs \ln (MO_2) relationships were plotted for each salinity level in each trial, and the equation of the line fitted by ordinary least-squares (OLS) regression in Minitab (version 15). This was used to predict MO_2 at standard masses for each species. A 95% confidence interval was used for these fitted lines at the standardised masses, calculated following Bailey (1995), to represent typical variability around the fitted values. Plotting these standardised values made it more tractable to visually compare the differences in response at each salinity for different sizes of individual in each species (see figs. 2.2 to 2.7).

Exploratory analysis using Kolmogorov-Smirnov tests on residuals of full factorial models indicated that the best conformity to ANOVA assumptions were achieved using log transformed MO_2 and body mass data ($R^2 = 0.63$). The main analysis was then conducted in three parts. The first level comprised the entire data set, with all species, trial durations and salinities being considered, to test whether all species have

2.2. MATERIALS AND METHODS

the same oxygen consumption across salinity, duration or mass. An ANOVA General Linear Model (GLM) was used (Minitab 15.0). The model used species and trial duration as fixed factors, with salinity as a random factor nested within duration (not all salinities were tested over both durations), and $\ln(\text{mass})$ as a covariate. Slopes of $\ln(\text{mass}) - \ln(\text{MO}_2)$ relationships across salinities, durations and species comparisons were checked for homogeneity (indicated by non-significant factor \times covariate terms in the full mixed model) prior to analysis of slope elevation. All ANOVAs were checked for issues of deviation from assumptions by testing unstandardised residuals for deviation from normality using Kolmogorov-Smirnov tests. When the slopes were found to be homogeneous, the analysis was run again, removing the $\ln(\text{mass}) \times$ factor interaction terms. It was then possible to see the direct comparisons of salinities and where, if any, significant differences lie, using Tukey's *post-hoc* tests to test for significant pair-wise differences between challenge salinities. However, as the individual variation of mass and response of organisms differed across salinities this result only gives an indication of likely between-salinity effects.

The second level of analysis was conducted using single species with both durations pooled. This was to test whether the oxygen consumption within individual species was the same across salinity, duration or mass. Again, the ANOVA GLM protocol was used, with the same fixed and random factors and sequence of tests, with the analysis being run on each species individually. The third level of analysis was conducted using single species and separating out the different durations into separate analyses. This tested whether the oxygen consumption of an individual species was the same across salinity or mass. The model used in this instance was simplified, with the fixed factor being salinity and the covariate $\ln(\text{mass})$. If the slopes were homogeneous for

2.3. RESULTS

the interaction between salinity and $\ln(\text{mass})$, then $\ln(\text{mass})$ was removed as a main factor in the model, but remained as a covariate. The analysis then proceeded using the same general approach as before.

2.3 Results

The results of the analysis showed that the species MO_2 were not significantly different from one another (see table 2.1). Even after mass was removed as a main factor in the model, which remained as a covariate, there was still a strong link between body mass and MO_2 . MO_2 differs significantly according to what species, length of exposure and salinity combination were being tested with respect to mass. MO_2 was also modified by species and duration of trial. In order to further understand the main factors causing the significantly different MO_2 , the data were split to test each species individually.

Table 2.1: Results of ANCOVA analysis of salinity effects upon MO_2 in all three amphipod species including both durations and all test salinities (© indicates covariate)

Source	DF	MS	F	P
Species	2	0.606	1.520	0.221
Duration	1	< 0.001	0.000	0.990
Species x duration	2	0.215	0.540	0.583
Salinity (species x duration)	25	0.837	2.560	<0.001
$\ln \text{mass}$ ©	1	20.108	61.620	< 0.001
$\ln \text{mass} \times \text{salinity}$ (species x duration)	25	0.645	1.980	0.005
Error	270	0.362		

2.3. RESULTS

2.3.1 *C. volutator*

The MO_2 for *C. volutator* did not differ significantly between trial durations. However, in certain salinities the relationship with duration changes and results in significantly different MO_2 s ($P < 0.001$) (see table 2.2). A significant result was found for the interaction between salinity within duration and mass, therefore, mass was left in the model as a factor. The analysis was run again separately for the short and medium term data.

Table 2.2: Results of ANCOVA analysis of salinity effects upon MO_2 in *C. volutator* including both durations and salinity exposure (© indicates covariate)

Source	DF	MS	F	P
Duration	1	0.694	0.700	0.414
Salinity (duration)	13	1.288	4.250	< 0.001
In mass ©	1	49.853	1.64570	< 0.001
Duration x In mass	1	0.077	0.250	0.616
Salinity(duration) x In mass	13	1.051	3.470	<0.001
Error	138	0.303		

When the durations were tested separately, significant differences in MO_2 were found at different salinities and masses (see table 2.3). However, for the short term data, it was not possible to run the Tukey tests due to the complication of mass as an interacting main effect in the model. A complex interaction was found between salinity and mass, which made the significant differences noted in the MO_2 difficult to interpret (see fig 2.2).

The Tukey tests were possible for the medium term data and showed that the MO_2 at 25 ppt was significantly higher compared to 10 and 5 ppt (see fig. 2.3; table 2.3).

2.3. RESULTS

The lack of a significant interaction between salinity and mass in the medium-term data might indicate acclimation. However, this results must be taken into context against the significant MO_2 results found for salinity alone. This would indicate that *C. volutator* has different respiration rates at different salinities, and might only acclimate at mid-range salinities (see fig. 2.3). This could be reflective of acute shock experienced at both time durations when the animals were exposed to a change in salinity.

Table 2.3: Results of ANCOVA analysis of salinity effects upon MO_2 in *C. volutator* after 4 hour and 5 day salinity exposure (©indicates covariate).

Source	DF		MS		F		P	
	4 hr	5 day	4 hr	5 day	4 hr	5 day	4 hr	5 day
ln mass ©	1	1	24.602	25.284	70.480	112.750	<0.001	0.078
Salinity	8	5	1.796	0.475	5.140	2.120	<0.001	<0.001
Salinity x ln mass	8	5	1.646	0.099	4.720	0.440	<0.001	
Error	87	51	0.349	0.224				

2.3. RESULTS

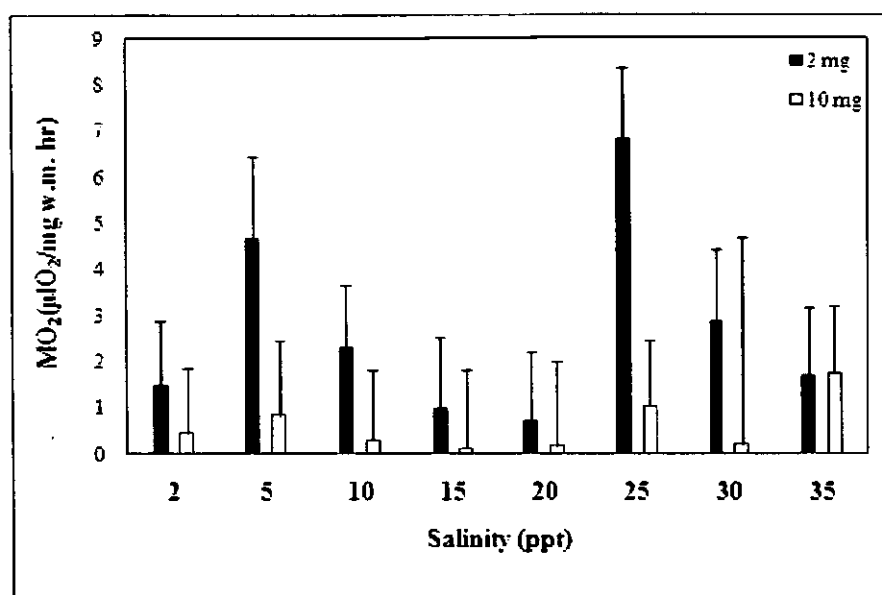


Figure 2.2: Mean oxygen consumption (MO_2) for *C. volutator* over short term duration (4 hours) for two size classes ($\pm 95\%$ CI).

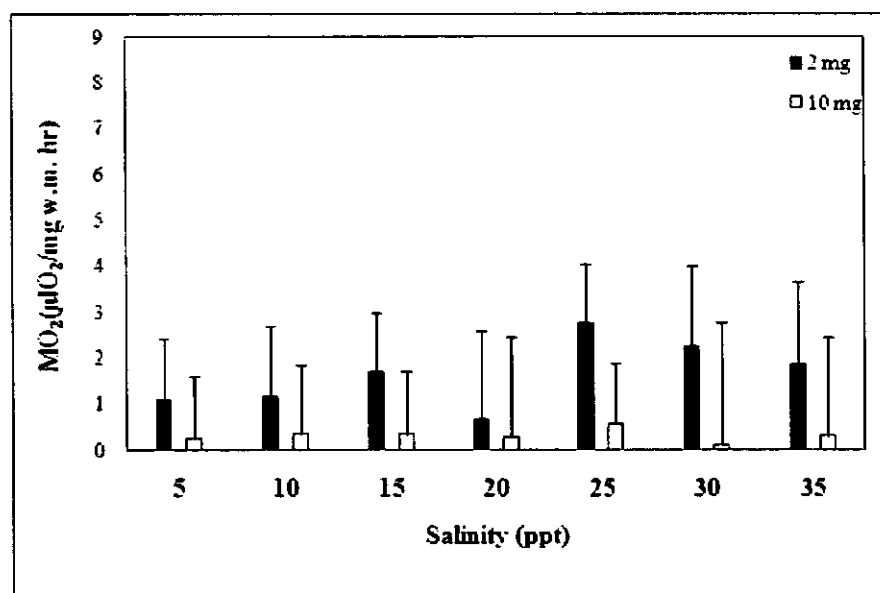


Figure 2.3: Mean oxygen consumption (MO_2) for *C. volutator* over medium term (5 day) duration for two size classes ($\pm 95\%$ CI). Note: Scale adjusted to figure 2.2 to aid comparison.

2.3. RESULTS

2.3.2 *G. marinus*

All factors were found to have significant effects on MO_2 (see table 2.4), after removal of mass as a main factor in the model. In order to see where these effects lie, the durations were separated and a further analysis conducted.

Table 2.4: Results of the ANCOVA analysis of salinity effects upon MO_2 in *G. marinus* including both durations and salinity exposure (© indicates covariate).

Source	DF	MS	F	P
Duration	1	0.011	0.090	0.768
Salinity (duration)	5	0.033	0.200	0.962
In mass ©	1	3.528	21.390	< 0.001
Duration x In mass	1	0.010	0.060	0.805
Salinity x In mass (duration)	5	0.016	0.100	0.992
Error	66	0.165		

The MO_2 for *G. marinus* over the short-term duration, was significantly higher at low compared to high salinities (with mass as a covariate only) (see table 2.5 and fig. 2.4). The MO_2 at 2 ppt was significantly higher than those of 20 and 35 ppt. Likewise the MO_2 found at 5 ppt was significantly higher than 20 ppt.

There does appear to be some acclimatory ability in the medium-term MO_2 results, as the response was not significant even after being tested without mass (see table 2.5 and fig. 2.5). The differences lie in the metabolic rate of larger individuals being different to much smaller individuals within the species and not salinity. What should be noted though, was that there are no data available for 2 ppt in the medium term experiments.

2.3. RESULTS

This was due to the high mortality experienced when *G. marinus* was exposed to very low salinity for extended periods of time.

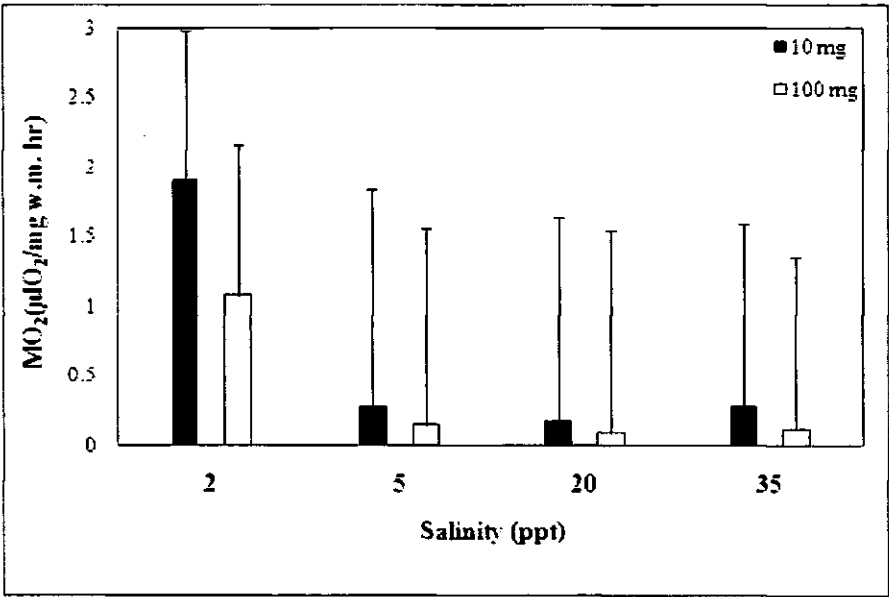


Figure 2.4: Mean oxygen consumption (MO_2) for *G. marinus* over short term duration (4 hours) for two size classes ($\pm 95\%$ CI).

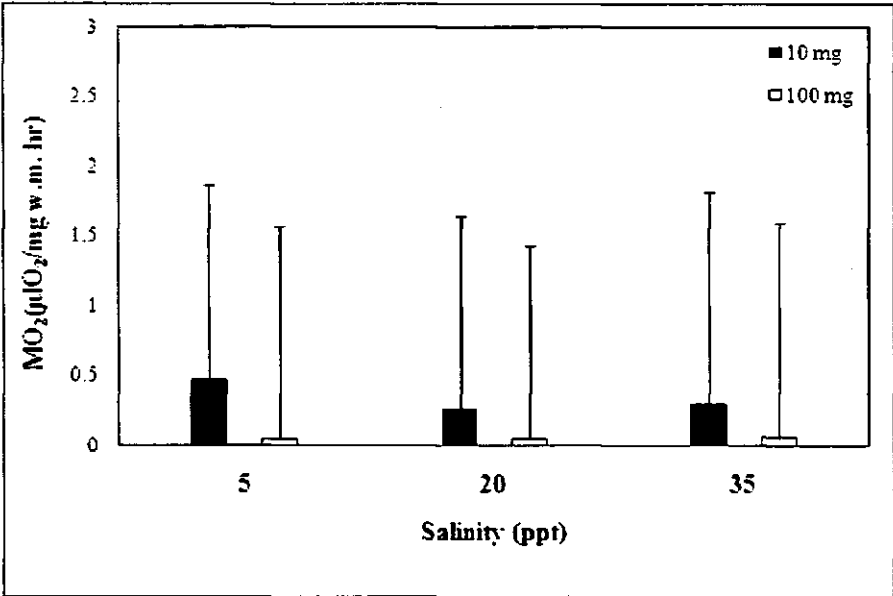


Figure 2.5: Mean oxygen consumption (MO_2) for *G. marinus* over medium term duration (5 days) for two size classes ($\pm 95\%$ CI).

2.3. RESULTS

Table 2.5: Results of ANCOVA analysis of $\ln \text{MO}_2$ of *G. marinus* over 2 durations (4 hours and 5 days) and various salinities (see text for details) (@ indicates covariate).

Source	DF		MS		F		P	
	4 hr	5 day	4 hr	5 day	4 hr	5 day	4 hr	5 day
$\ln \text{mass}$ @	1	1	1.346	14.681	8.580	96.540	0.006	<0.001
Salinity	3	2	1.764	0.404	11.250	2.660	<0.001	0.084
Error	36	35	0.157	0.152				

2.3.3 *G. chevreuxi*

Similar results to *G. marinus* were seen for *G. chevreuxi*. Significant interactions between MO_2 and all factors were found (see table 2.6). As before, the short-term and medium-term data were separated as there were significant differences in the MO_2 depending on the duration. Mass was removed as a main factor in the model when no significant interactions were found.

2.3. RESULTS

Table 2.6: Results of ANCOVA analysis of salinity effects upon MO_2 in *G. chevreuxi* including both durations and salinity exposure (© indicates covariate).

Source	DF	MS	F	P
Duration	1	0.194	0.360	0.550
Salinity {duration}	7	0.575	1.070	0.392
ln mass ©	1	3.562	6.640	0.012
Duration x ln mass	1	0.509	0.950	0.334
Salinity{duration} x ln mass	7	0.342	0.640	0.724
Error	66	0.537		

For the short-term data (see table 2.7, fig. 2.6), there was a significant effect of salinity, with the MO_2 found to be higher at 35 ppt than 15 or 20 ppt. However, despite removal of mass as a main factor in the model, examination of residuals for this analysis reveals marginal conformity to assumptions of homogeneity of variance.

The respiration rate of *G. chevreuxi* over the medium-term duration, was considerably more variable than that of *G. marinus* (see figs. 2.6 and 2.7). However, there was no significant difference found between the MO_2 for the salinity trials of *G. chevreuxi* ($P = 0.397$), which is notable as compared to *G. marinus* ($P = 0.084$). This indicated that *G. chevreuxi* has the greatest acclimatory ability over the 5 day period and that the resulting difference in respiration rates were due to differences in mass of the individual animals. However, acclimation may be occurring but the residuals deviate significantly from normality and were considered marginal; therefore these results cannot be thought of as conclusive.

2.3. RESULTS

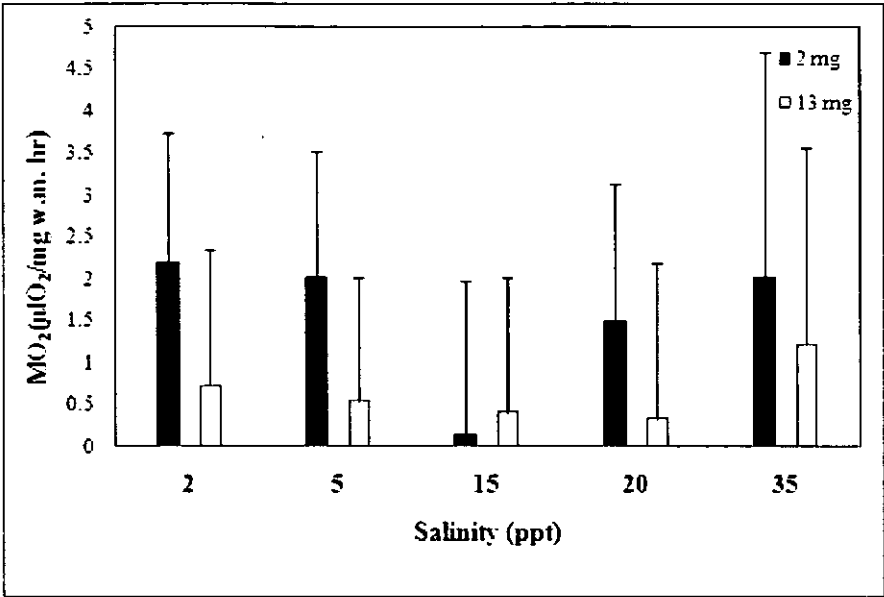


Figure 2.6: Mean oxygen consumption (MO_2) for *G. chevreuxi* over short term duration (4 hours) for two size classes ($\pm 95\%$ CI).

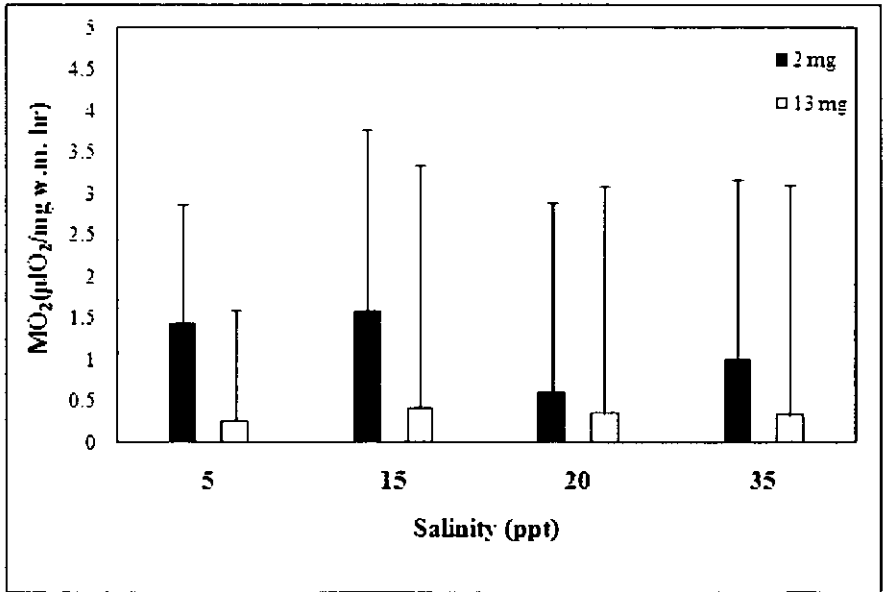


Figure 2.7: Mean oxygen consumption (MO_2) for *G. chevreuxi* over medium term duration (5 days) for two size classes ($\pm 95\%$ CI).

2.4. DISCUSSION

Table 2.7: Results of ANCOVA analysis of $\ln \text{MO}_2$ of *G. chevreuxi* over 2 durations (4 hours and 5 days) and various salinities (see text for details) (©indicates covariate).

Source	DF		MS		F		P	
	4 hr	5 day	4 hr	5 day	4 hr	5 day	4 hr	5 day
$\ln \text{mass}$ ©	1	1	10.545	13.214	25.980	19.730	<0.001	<0.001
Salinity	4	3	1.918	0.683	4.730	1.020	0.003	0.397
Error	45	31	0.406	0.700				

2.4 Discussion

In general, the amphipods had significantly different MO_2 s and this was being modified by the interaction between salinity and duration. This was to be expected as the three species have distinctly different habitats.

2.4.1 *C. volutator*

It was predicted that the estuarine amphipod *C. volutator* would be better adapted to salinity variation than the marine *G. marinus*. This assumption was not upheld; the indication was that *C. volutator* does not acclimate to salinity changes and responds to the stresses of a change in salinity by increasing its rate of oxygen consumption which may have significant energetic cost. *Corophium volutator* appears to be limited by the increased physiological demands when there is a change in salinity, which was reflected in the strongly significant result for salinity in the short term trials. The significant result of the interaction between salinity and mass indicates that different sized individuals react differently to a change in salinity. The lack of acclimation

2.4. DISCUSSION

shows that *C. volutator* has a limited salinity range and that this is different for smaller individuals than for larger ones.

The highest oxygen consumption for the medium term experiments for *C. volutator* were at 25 ppt for both standardised masses. This might indicate a threshold salinity, beyond which the animals need to shift energy requirements in order to survive. These results are different from those of previous research by McLusky (1969), where he found no change in respiration rate at differing salinities for *C. volutator*. However, McLusky (1969) does contextualise the results as previous research (Potts 1954, McLusky 1967), by demonstrating that *C. volutator* can produce hypo-osmotic urine. These findings as well as the possibility of shifting energy expenditure away from growth and reproduction in order to survive in the changing salinity, can explain the difference in results. Harris & Musko (1999) found differing rates of oxygen consumption for *Corophium curvispinum* (Sars) depending on whether or not the animal was in its burrow or not. This is significant as in the current experiment, animals were put in to darkened vials with a small amount of mesh and considered to be at rest. In order to achieve standardised methodology for *C. volutator* and the gammarids, it was not possible to use natural burrows, which might have added further variability to the experiments, as the bacteria in burrows are known to have high O₂ consumption rates (Harris & Musko 1999). Previous research comparing the free-living *Marinogammarus obtusatus* (Dahl) and two species of tube-dwelling *Corophium* showed different ventilatory strategies (Gamble 1970). Hyperventilation at low oxygen concentrations occurred at higher temperatures in *M. obtusatus* but not in the *Corophium* species. Both the *Corophium* species increased the frequency of pleopod beating at higher temperatures but not the speed. The pleopods of the *Corophium* species start to beat earlier

2.4. DISCUSSION

at higher temperatures, but this has a cost in terms of increased metabolic rate (Gamble 1970). This type of increased ventilation could also account for some variability in the data in that *C. volutator* might change the rate of pleopod beating depending on the salinity creating a corresponding change in respiration rates.

2.4.2 *G. marinus*

In the short term experiments *G. marinus* shows the classic negatively exponential trend of high respiration rates at extremely low salinities, tapering off as the salinity increases to full seawater. The *Fucus* species which *G. marinus* are found in association with are most often found in fully marine to brackish waters (Lincoln 1979). Hence, the short-term physiological response to changes in salinity are reflective of the distribution of this species. Smaller individuals were seen to have a higher MO_2 regardless of duration, and that it was the mass of the individual which was driving the relationship. Dorgelo (1977) showed that *G. marinus* has the widest salinity tolerance at 5°C, with the preference narrowing with increasing temperature. Despite its theoretical marine origin, *G. marinus* appears to have the lowest MO_2 at 20 ppt in the short term experiments, which might be an indicate a better metabolic capacity reflective of a life in an estuarine environment. The lack of a significant result for the medium term data indicates an unexpected acclimatory ability of *G. marinus*. What can be drawn from this work is that this species is not limited by physiological constraints, rather, it is more likely limited by habitat availability.

2.4.3 *G. chevreuxi*

The variability in the *G. chevreuxi* data are important to note as the analysis shows some possible experimental artifacts within the data as seen with the marginal con-

2.4. DISCUSSION

formity of the residuals. Yet, overall it appears that this species exhibits acclimation during the medium term experiments, as it has significantly lower MO_2 than during the short term experiments. This may indicate that their habitat is not as stable as predicted, or that they live well within their physiological limits and can sustain large changes in environmental conditions without needing to change distribution. The data also show a preference for lower salinities as the MO_2 during the completely marine trials are higher than those found for 20 ppt. Previous research found a decrease in oxygen consumption when *G. chevreuxi* was transferred from low to full strength salinity (Sutcliffe 1984, Lowenstein 1935). This same pattern also appears to be partially replicated here, with highest MO_2 at the lowest salinities (2 and 5 ppt) in the medium term experiment. For the short term experiments, *G. chevreuxi* appears best adapted to 15 ppt, with increased MO_2 both at higher and lower salinities.

2.4.4 Conclusions

This work demonstrates the need to carefully consider the impact of mass on MO_2 , as there are conflicting results on the relationship between mass and oxygen consumption. Some relationships are positive and others negative (Sutcliffe 1984). Juveniles are considered to have a very high rate of activity and therefore require more oxygen (Sutcliffe 1984). The opposite is true, for older animals, their rate of oxygen uptake can be greatly depressed (Sutcliffe 1984). It is possible that either a positive or negative relationship can occur which is defined by the life stage, and particularly with the great increase in mass associated with adult versus juvenile amphipods, as is seen in the current research. In outright terms, larger individuals consume more oxygen but when mass is considered, larger individuals consumer less oxygen per mg. This might be due to the surface area:volume ratio, or due to the higher energetic demands

2.4. DISCUSSION

of growth in smaller individuals. Further complicated by this, is life stage and reproduction state of adult females, as production and brooding of eggs has metabolic costs (Morritt & Spicer 1996^{b,c}, Spicer & Stromberg 2003). Hence, the relationships between mass and oxygen consumption is not simple and should be considered carefully for individual experiments.

If the lowest oxygen consumption is a true reflection of an animal's condition, then all three species show optimal performance at the mid-range salinity between 10 and 25 ppt. These data, particularly for the *Gammarus* species, appear to support the idea that there can be true estuarine species, rather than freshwater or marine animals living at their physiological extremes (Attrill & Rundle 2002). These species did not follow the expected trend of increasing oxygen consumption with increasing size, rather it was the smallest individuals which were shown to have the highest MO_2 per mass. This has important implications for these species distribution. Smaller individuals are, at least in part, driving the distribution of these three species of amphipods. This is because the smaller individuals have a more restricted physiological window and when stress is induced by a change in salinity, the potential exists for the population to be reduced. Increased oxygen consumption can be energetically costly and these increases in MO_2 are seen to occur in fully marine or close to freshwater conditions. The narrow salinity range as defined by the respiration rate of *C. volutator* might explain some of the patchy distributions noted previously (e.g. Lawrie et al. 2000), as spring tides can change a site from brackish to fully marine in a short space of time. The classification of *G. chevreuxi* as a rare species (Lincoln 1979) might simply be a reflection of its limited habitat niche, as its strong acclimatory ability to changes in salinity does not appear to be driving its distribution, much like the patches of *Fucus* appear to limit *G. marinus*.

Chapter 3

Effects of biotic and abiotic factors on physiological tolerances

3.1 Introduction

Distribution of species is rarely determined by a single environmental factor, it is often shaped by a multitude of aspects (Somero 1997, Gaston & Spicer 1998, Braby & Somero 2006). Nonetheless, niche segregation has been linked with temperature tolerance and has been shown to be a determinant of distribution of species (Gaston & Blackburn 2000). The importance of temperature is even more pronounced in a variable environment such as an estuary (Re et al. 2005). In general, it is considered that more widespread species have a greater thermal tolerance than those with a narrow range (e.g. Calosi et al. 2008). Yet this is does not necessarily hold true in the southern hemisphere for such groups as lotic Chironomidae, due to a less predictable temperature and flow regime within rivers (McKie et al. 2004). Despite the complex nature of the factors which determine a species' niche, temperature can be considered important when trying to understand both local and large scale distribution. In order to make predictions in a changing climate about the distribution and persistence of amphipods in their present habitats, it is important to consider not just their physiological performance under current environmental conditions, but also their absolute tolerances, as

3.1. INTRODUCTION

determinants of their thermal niche (Rodriguez et al. 1996, Cowling et al. 2003). In order to determine the extent of the impact of biotic and abiotic factors on thermal biology, manipulative experiments are required (e.g. Cowling et al. 2004). Comparing several related species which have discrete niches can elucidate the possible drivers of distribution (Hoffmann & Blows 1994). This idea was applied here, as *C. volutator* lives on mud flats which are adjacent to flowing rivers that are inhabited by *G. chevreuxi* which are located next to discrete patches of *Fucus* spp. where *G. marinus* can be found. Heat shock or critical thermal maximum (CTMax) as well as critical thermal minimum (CTMin) are both parameters which have the potential to be key limiting factors in a species' distribution. Further to this aim, incorporating salinity as well as seasonality has the potential to reveal interactions between factors that impact on the distribution of these amphipods.

There is a tremendous amount of research available on thermal tolerances of organisms, but most concentrate on vertebrates with only a small number on invertebrates (Lagerspetz & Bowler 1993), with fewer still on specific groups of crustaceans (Kinne 1970). A search of recent scientific literature reveals only a few papers focusing on amphipods and thermotolerance. Some examples include the upper thermal tolerance of the invasive amphipod, *Arcitalitrus dorrieni* (Hunt) (Cowling et al. 2003). Lahdes & Vainio (2003) meanwhile studied upper thermal tolerance in Baltic amphipods, *Gammarus* spp., which showed seasonal trends in heat maxima of just below 33°C in the summer and 30.2°C in the autumn, indicating that seasonal acclimation may occur in these species. Further review of the literature reveals older research on the thermal tolerance of amphipods. For example, Buchanan et al. (1988) found the freshwater amphipod, *Paramelita nigroculus* (Barnard), to have a CTMax between 33.4 and 35.3°C,

3.1. INTRODUCTION

and a higher thermal tolerance was noted when animals were acclimated to 20°C as compared to 8.5°C. The beach-hopper, *Orchestia gammarellus* (Pallas), was found to have a wide temperature tolerance between 0 and 30°C (Moore & Francis 1986). Owing to a lack of specific recent research therefore, the basis for the work presented here must draw upon examples from literature concerning differing species.

Temperature Several species of dogwhelks were tested for high temperature tolerance by Sorte & Hofmann (2005). Those species found in southern and mid-latitudes at high tidal heights had better thermal tolerance in the form of reattachment capability, even after extended exposure to high temperatures. Those from mid (low tide height) and low latitude (high tide height) were significantly less thermotolerant, irrespective of acclimation (Sorte & Hofmann 2005). This study concluded that both the presence of heat shock proteins (Hsp 70) and physiological acclimation were responsible for the difference in temperature tolerances (Sorte & Hofmann 2005). A similar acclimatory ability was also seen in two species of *Desmognathus* salamanders (Zweifel 1957). Animals kept at 15°C had a higher CTMax than those acclimated to 5°C, but at the lower temperature acclimation, the salamander with a wider distribution (*D. fuscus* (Green)) was shown to have a higher CTMax compared to the more restricted *D. quadramaculatus* (Holbrook).

In general, animals acclimated to lower temperatures show significantly lower CTMax values. Frisbie & Lee (1997) found greater than expected cold temperature tolerance in several species of freshwater invertebrates. These animals were able to survive being exposed to temperatures ranging between -4.7°C to -7.9°C in dry conditions and down to -2.2°C in wet (in contact with ice) (Frisbie & Lee 1997). A lower tolerance to cold temperatures was seen in both these studies when the animals were

3.1. INTRODUCTION

exposed to ice formation (Frisbie & Lee 1997, Issartel et al. 2006). This has important implications for both the gammarids and *C. volutator* in the present study, especially males which display a searching behaviour associated with reproduction (Forbes et al. 1996) during which they crawl on the surface of the sediment. It is therefore possible that male *C. volutator* could experience a sudden drop in temperature, which would expose that portion of the population to increased mortality (J.I. Spicer pers. comm.).

Salinity Much of the literature concerning thermal tolerance with respect to salinity deals with temperature and salinity as separate factors (e.g. Cowling et al. 2003, Moore & Francis 1986). For example, Cowling et al. (2003) worked extensively with the terrestrial amphipod, *Arcitalitrus dorrieni*, with respect to humidity, CTMin, CTMax and salinity. However in this study, each factor was dealt with separately; whilst this is favourable for simple analysis it does not necessarily reflect the complex natural environment, in which factors such as temperature and salinity may co-vary frequently. In much earlier work, the marine gastropod, *Murex pomum* (Gmelin) was found to have a relatively narrow range of lethal temperatures between 10 and 33°C, with salinity tolerance being between 10 and 50 ppt (Sander & Moore 1979). However, this work did not look at the interaction between the two factors. More recent research on blue crabs *Callinectes sapidus* (Rathbun) does address the interaction between temperature and salinity (Rome et al. 2004). These crabs have a marked increase in mortality when low salinity and low temperatures are combined. Further to this, the crabs' mortality increases when a sudden drop in temperature occurs, indicating a lack of short term tolerance to low temperatures irrespective of salinity (Rome et al. 2004). This research demonstrates the importance of the interaction between salinity and temperature tolerance. Although this work goes some way to addressing the issue of combined factors,

3.1. INTRODUCTION

it focuses on lethal limits rather than more ecologically valid endpoints (see methodology).

Seasonality Measuring thermal tolerance of animals in the laboratory is an important tool for understanding the range limits of a species. This may, however miss the more complex underlying factors which drive the behavioural and physiological responses of the species by removing important factors such as seasonality. The ability of an animal to tolerate sudden temperature change has been seen to be modified by the temperature previously experienced as shown in the amphipod *G. limnaeus* (Smith) (Krog 1954). In this research, animals collected in the summer were able to survive for one hour between 30 and 32°C. This is in contrast to animals collected in the winter which showed a maximal thermal limit of 26°C (Krog 1954). Recent research on lizards has shown seasonality of body temperature regulation noted in *Microlophus atacamensis* (Donoso-Barros), a wide spread species. These data conflicted with previous research (Vidal et al. 2002) on the same species. However, the research of Vidal et al. (2002) may have missed potential patterns due to their experiments being conducted mostly in the winter, leaving the results questionable due to the relevant effect of seasonality.

3.1.1 Methodology

The need to develop and use a standard methodology is one which affects many disciplines. In the case of thermal biology, the inability to compare across results in studies using CTMax and CTMin as an experiment's 'endpoint' can arise over differences in interpretation of CTMax and CTMin and the exact definition of the 'endpoint'. The original definition of CTMax was given by Cowles & Bogert (1944) as: 'the thermal point at which locomotory activity becomes disorganised and the animal loses its abil-

3.1. INTRODUCTION

ity to escape from conditions that will promptly lead to its death'. Many papers have used this endpoint, implying that death itself is not necessarily the only meaningful endpoint. From an ecological perspective for example, the point where an animal can no longer escape threat (Lowe & Vance 1955) or where the animal becomes clearly handicapped (Rodriguez et al. 1996) can also be a valid endpoint. A review of the literature and further research conducted by Lutterschmidt & Hutchison (1997a,b) found this reduction in escape ability to be widely interpreted and many different endpoints used depending on species and user preference. Rodriguez et al. (1996) encountered similar difficulties when comparing their research to previous work, as they consider CTMax to be a behavioural response and highly variable. Lutterschmidt & Hutchison (1997b) found that onset of spasms was significantly more precise an endpoint compared to loss of righting response in a variety of fish and reptile families. Manush et al. (2004) used a similar endpoint, where abdominal muscle segments contract, but this leads to inversion in the freshwater prawn *Macrobrachium rosenbergii*. As here, similar endpoints result in distinctly different physical manifestations. This research is useful but can be limited in its comparison as the precise endpoints compared by Lutterschmidt & Hutchison (1997b) simply do not occur in amphipods, however meaningful endpoint selection was considered throughout this study nonetheless.

The aim of this chapter is to measure the effect of biotic and abiotic factors upon physiological tolerances across the three amphipod species. This information provides valuable insight into intra- and inter-specific tolerance in the three species of amphipods, which will be related to spatio-temporal and demographic patterns in the final chapters of the thesis. Greater tolerance, especially amongst more susceptible life stages, is expected to confer greater spatio-temporal and demographic stability on amphipod

3.2. MATERIALS AND METHODS

populations, whilst those species with lower tolerances or lower capacities to acclimate to change might be expected to display more stochastic variability in these population level parameters if physiological capacity is indeed a determinant of population distribution and abundance. Here, the biotic factor investigated was body mass and the abiotic factors are temperature, season and salinity. It was expected that *G. chevreuxi* will have the narrowest thermal range, due to living in constantly flowing streams, with *C. volutator* exhibiting the widest thermal range due to its estuarine mud flat habitat with large temperature fluctuations from low to high tide. Finally, *G. marinus* was expected to have a thermal range between that of the other two amphipod species due to its potential for large temperature fluctuations at low tide which would be limited by its marine origins. Physiological tolerances are measured as CTMin, CTMax as well as the temperature range (ΔCT).

3.2 Materials and Methods

In order to elucidate if there is any thermal adaptation occurring between seasons as well as testing the impact of acclimation temperature, salinity and mass, these experiments were conducted in two parts. The first part comprises trials using animals collected during the summer and the second animals collected during the winter.

Corophium volutator were collected from the Avon estuary (National grid reference SX 666 439) and *G. marinus* and *G. chevreuxi* from the Plym estuary (National grid reference SX 540 566), first in September 2007 and again in February 2008. All life stages (juveniles, females in various modes of production and males) were used in this experiment. The reason for this is that all life stages must be able to adapt to the changing thermal regime (Re et al. 2005), or this will result in limitations of the reproduction

3.2. MATERIALS AND METHODS

or survival of the population. Collected animals were returned to the laboratory and kept at 20 ppt for *C. volutator* and *G. chevreuxi* and 35 ppt for *G. marinus*, in natural daylight and environmental ambient temperature of 15°C for two days. Thereafter they were split into two groups, each of which was housed in a controlled temperature room with 12:12 light cycle; one group was maintained at 10°C and the other at 20°C. The animals were left to acclimate in these conditions for two days before being divided once again into three different salinities in each temperature regime. To cover the entire range of salinity, animals were kept in 2, 20 and 35 ppt. Animals were left to acclimate for 2 days before experiments commenced.

All experiments were conducted using Grant LTC water baths (Grant Instruments, Cambridge, England). There were 12 animals used per test salinity and acclimation temperature combination. For CTMax experiments, animals were placed in individual 15 mm diameter wells (see fig. 3.1) in the same salinity, and at the same temperature they had been acclimated to. The temperature was then increased at a rate of 1°C/minute. The animals were monitored throughout the experiment and 4 endpoints were found to be consistent across all three species:

- 1) Stop swimming,
- 2) Stop beating pleopods - pleopods do not restart beating, even when prodded,
- 3) All movement ceased - no movement at all even when prodded,
- 4) Death - no movement occurs even when removed from the heat.

3.2. MATERIALS AND METHODS

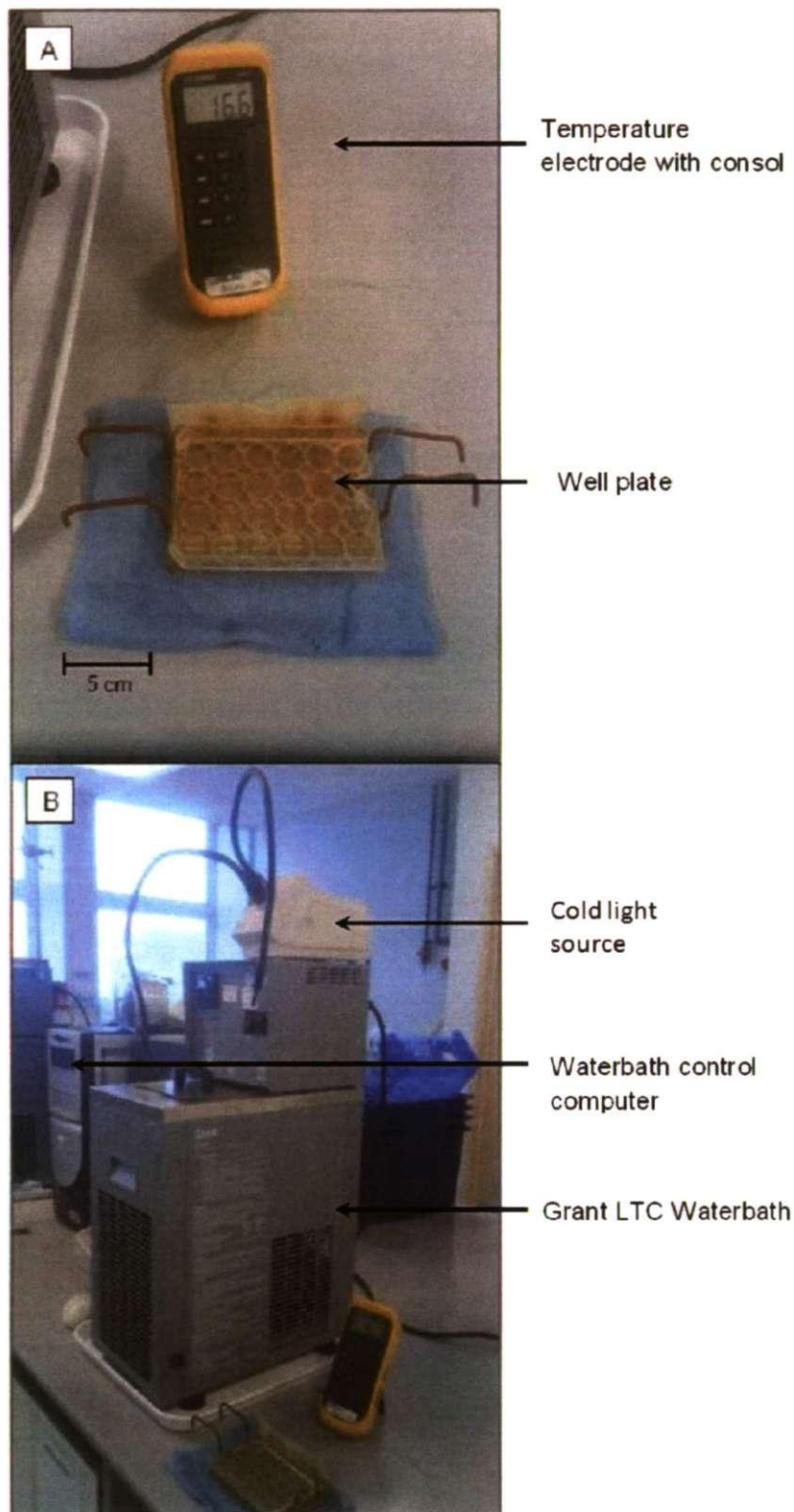


Figure 3.1: Experimental set-up (a) electronic thermo-couple with the modified 16 cell plate where individual animals were held and (b) the Grant water baths

3.2. MATERIALS AND METHODS

For the CTMin experiments, animals were blot dried and trials conducted in dry chambers in the air to avoid ice-formation prior to the animals reaching endpoints. *Corophium volutator* had four endpoints revealed through preliminary study:

- 1) Stop climbing - *C. volutator* stopped climbed the side of the well.
- 2) Stop movement - *C. volutator* would continue to search after stopping climbing and the endpoint was reached when all movement ceased.
- 3) No movement when prodded - Animals do not move when prodded.
- 4) No movement after warming - Animals were removed individually and held in the centre of the hand. A finger gently covered the animal for 3 second. If movement did not start immediately, the animal was considered dead.

Due to the differing behaviour of the *Gammarus* species, they do not climb and remain still regardless of the temperature, thus only endpoints 3 & 4 were used in these cases.

Once the experiments were completed, animals were blot dried and weighed (Fisher-brand PS-100 precision balance) and put into individual Eppendorf tubes (vol. = 5 mL) with 70% alcohol. When all experiments were completed, each individual animal was sexed on a Meji microscope and lengths determined using a Dino-lite digital microscope. Measurements were made from between the eyes to the tip of the telson.

3.2.1 Statistical Analysis

The results of the thermal limit experiments were analysed using Minitab (version 15). Data were analysed using an ANOVA approach in a General Linear Model (GLM) using three factors: salinity, acclimation temperature and season. Body mass was used as a covariate after first testing for homogeneity of covariate slopes across all factor

3.2. MATERIALS AND METHODS

combinations (Underwood 1997). If the slopes were found to be parallel, mass was removed from the model but remained as a covariate. The slopes of the relationship between mass and CT data were found to be parallel for all instances.

The results of the CTMax and CTMin experiments were analysed separately. For each temperature extreme, the analysis was conducted in four parts. First, all data were pooled to check for statistical differences between species across acclimation temperature, season and salinity. Next, the endpoint of death was used in the preliminary analysis, to test the entire dataset against all factors to determine if there were significant differences between species or if there was a single factor driving the endpoint temperature. Bonferroni simultaneous tests for pairwise comparisons were conducted for the three species and test salinities to see where significant differences, if any, lay. To ensure the most pertinent data are analysed (see section 3.1.1), the ecologically relevant endpoint of 'stop swimming' for CTMax and 'no movement when prodded' for CTMin, were used as the focus for both of the remaining two parts of the analyses. This is because even if animals continued to survive, they cannot escape predation, resulting in death.

Next, the *Corophium* data were removed and the analysis was run again with just the *Gammarus* spp., to see whether the two species differed significantly in their endpoint temperatures, across the acclimation temperature, season and salinity.

The final part of the analysis was to run the GLM protocol on each species separately. This was to test which factors had significantly different endpoint temperatures, and whether any factors were interacting significantly. Tukey pairwise comparison tests were conducted on the test salinities for each species to check for significant differences in endpoint temperatures.

3.3. RESULTS

Three life stages were considered for all three species: males, females and juveniles. *C. volutator* males were distinguished by their small penial papillae at the base of the 7th pair of legs (Schneider et al. 1994) and large spine on their antenna, and females by the smaller antennal spine and (where present) oostegites. Male gammarids were distinguished from females by the size and shape of their gnathopods and where present, oostegites. Because life stage could only be determined after the end of the experiment, some combinations of life stage, season, salinity, acclimation temperature were inadequately represented for the GLM to proceed. Factors were therefore removed sequentially based on their main effects. Juveniles were categorised in *C. volutator* and *G. chevreuxi* as individuals less than 2 mm in length (from between the eyes to the start of the telson) and less than 3 mm in length for *G. marinus*.

Thermal range Comparing the various temperature and salinity combinations across mass during the summer and winter is difficult. For this reason, best-fit linear trendlines were fitted for each temperature and salinity combination in Microsoft Excel. The resulting equations were used to produce CTMax and CTMin data across standardised masses, based on the maximum and minimum from the CT data, for *C. volutator* and *G. chevreuxi* were between 0.0001 and 0.015g, and for *G. marinus* 0.0001 and 0.25g. The Δ CT was then plotted for all combinations.

3.3 Results

For ease of interpretation, the results were broken down into sections. The overall results for CTMax from the analysis including all species will be presented first (section 3.3.1) including the ecologically relevant endpoint for all species pooled followed by the Gammarid data only, and the endpoint of death for all species pooled. This will be

3.3. RESULTS

followed by the results for *C. volutator*, *G. marinus* and finally *G. chevreuxi*. The next section (3.3.2) will follow the same format, with the results for the entire dataset for CTMin, again with both the endpoints of ‘no movement’ and death, followed by the analysis results for each species in turn.

3.3.1 CTMax

Ecologically relevant endpoint: ‘stop swimming’ The amphipods ‘stopped swimming’ at significantly different temperatures. However, season, salinity and acclimation temperature effected each species upper thermal tolerance differently (see significant interactions in GLM, table 3.1) making generalities hard to determine. This analysis was repeated on the data for the gammarids only, and these were also found to differ significantly in their CTMax and show several significant interactions between factors (see table 3.1). The results of the pairwise comparisons indicated that at the endpoint of ‘stop swimming’, *G. chevreuxi* differed significantly from *C. volutator* ($P = 0.001$) and *G. marinus* ($P = 0.015$). The pairwise comparisons also indicated that the CTMax for 2 ppt differed significantly from 20 ($P < 0.001$) and 35 ppt ($P = 0.024$). The data were then subdivided and the species analysed individually (see sections below specific to each species).

Endpoint of death: ‘no movement when prodded’ For the endpoint of death, there are several differences noted when compared to the ecologically relevant endpoint (see table 3.1). There are fewer significant interactions which could be indicative that the endpoint of death was more reflective of absolute thermal limits. Equally, these more conservative results could indicate that using the endpoint of death masks the ecologically relevant behavioural response by going beyond the animal’s physiological

3.3. RESULTS

limits. Acclimation temperature was found to have a significant effect on the endpoint of death, with salinity and season individually not having significant results. Further significant results were noted when the acclimation temperature and salinity were combined (see table 3.1). The results of the pairwise comparisons showed that there was no significant differences between the salinities, but all species were significantly different from one another ($P < 0.001$). No further analysis of these data was undertaken, as analysis focused on the more ecologically relevant endpoint.

Table 3.1: Results of the GLM for CTMax endpoint of 'stop swimming' for all three species, the results for Gammarids as well as the the endpoint of 'death'

Factor	'stop swimming'						'death'		
	All species			Gammarids			All species		
	DF	F	P	DF	F	P	DF	F	P
Mass	1	4.55	0.034	1	4.39	0.037	1	3.51	0.062
Species	2	8.03	<0.001	1	10.09	0.002	2	109.80	<0.001
Season	1	37.79	<0.001	1	46.71	<0.001	1	1.84	0.176
Salinity	2	11.29	<0.001	2	3.87	0.022	2	0.69	0.503
Temperature	1	352.26	<0.001	1	255.13	<0.001	1	80.19	<0.001
Species*season	2	12.03	<0.001	1	21.91	<0.001	2	3.10	0.046
Species*salinity	4	12.19	<0.001	2	23.84	<0.001	4	3.96	0.004
Species*temp	2	17.36	<0.001	1	39.63	<0.001	2	7.76	0.001
Season * Salinity	2	4.12	0.017	2	4.02	0.019	2	1.98	0.139
Season* temp	1	0.00	0.987	1	0.01	0.918	1	5.33	0.022
Salinity * temp	2	4.7	0.009	2	3.91	0.021	2	7.69	0.001
Season*salinity*temp	2	3.76	0.024	2	8.74	<0.001	2	3.68	0.026
Species*season*salinity	4	4.70	0.001	2	7.79	0.001	4	2.97	0.020
Species*season*temp	2	25.27	<0.001	1	58.83	<0.001	2	11.99	<0.001
Species*salinity*temp	4	7.21	<0.001	2	7.33	0.001	4	4.05	0.003
Species*season*salinity*temp	4	5.11	0.001	2	2.17	0.117	4	3.30	0.011
Error	365			239			365		

C. volutator All factors were found to significantly impact the upper thermal tolerance of *C. volutator* (see table 3.2). The pairwise comparisons showed that the endpoint temperature was significantly different at 20 ppt compared to 2 ($P < 0.001$) and 35 ppt ($P = 0.008$) (see fig. 3.2). CTMax values were higher at 20°C compared to 10°C regardless of season (see fig. 3.3 a and b), which indicates a loss of seasonality and

3.3. RESULTS

acclimation to the test temperatures. When the life stage of the animals were included in the analysis, there was inadequate data in each treatment for each of the three life stages. Therefore, the GLM was run with season and salinity lumped together. No effect of life stage was found (see table 3.3). To verify the result gained by lumping the data, the baseline salinity (20 ppt) data were analysed separately and the statistical results confirmed.

Table 3.2: Results of the ANOVA GLM for *C. volutator* CTMax for the endpoint of 'stop swimming'

Factor	DF	F	P
Mass	1	6.72	0.011
Season	1	4.83	0.030
Salinity	2	11.20	<0.001
Temperature	1	114.10	<0.001
Season * Salinity	2	3.31	0.040
Season* temp	1	0.00	0.999
Salinity * temp	2	8.42	<0.001
Season*salinity*temp	2	3.66	0.029
Error	125		

Table 3.3: Results of the GLM for *C. volutator* CTMax for the endpoint of 'stop swimming' including life stage

Factor	DF	F	P
Mass	1	0.45	0.505
Temperature	1	61.09	<0.001
Life stage	2	0.84	0.435
Temp*life stage	2	0.33	0.720
Error	131		

3.3. RESULTS

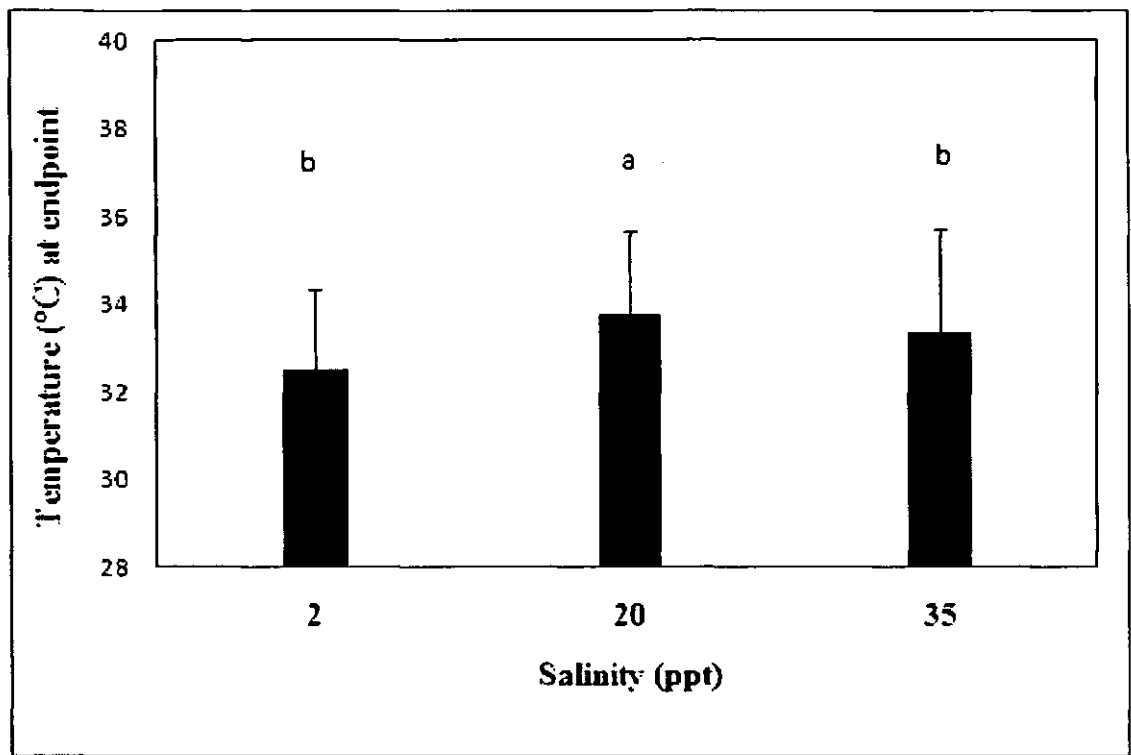


Figure 3.2: Average CTMax *C. volutator* temperature (°C) at endpoint of 'stop swimming' with acclimation temperatures (10 and 20°C) and season pooled. Error bars are standard deviations, letters are pairwise comparisons, number of data points (n) ranged between 35 and 54.

3.3. RESULTS

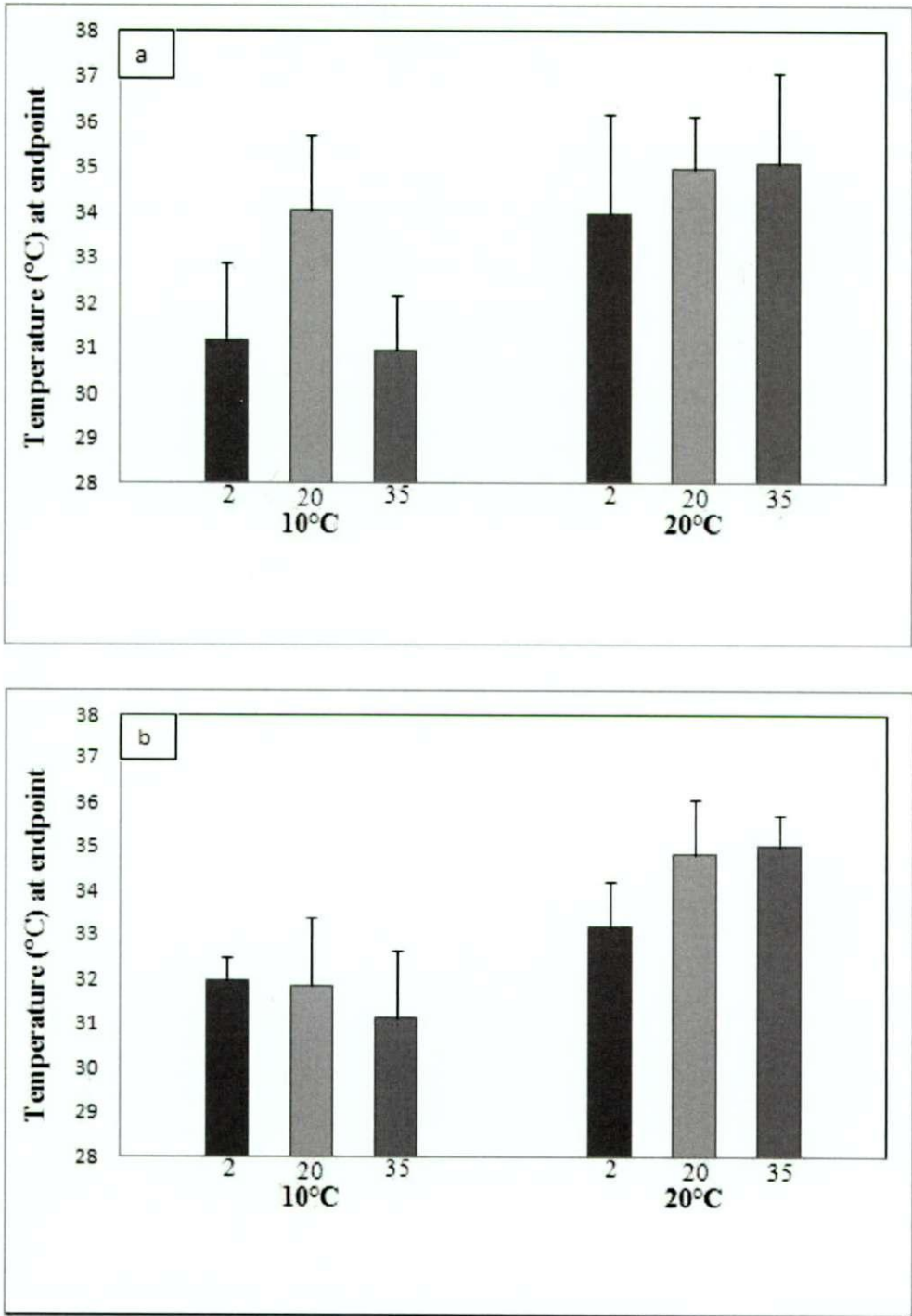


Figure 3.3: CTMax for *C. volutator* during the (a) summer and (b) winter treatments, temperature (°C) at endpoint of 'stop swimming' for each of the two acclimation temperatures (10 and 20°C) and each of the three salinities (2, 20 and 35 ppt). Error bars are standard deviations, number of data points (n) ranged between 5 and 17.

3.3. RESULTS

G. marinus All individual factors were found to significantly affect the CTMax of *G. marinus* (see table 3.4). The pairwise comparisons showed the endpoint temperature at 35ppt was significantly different to 2 ppt ($P<0.001$) and 20 ppt ($P<0.001$) (see fig. 3.4). Animals in the winter at 10°C, showed a significantly lower temperature at which they ‘stop swimming’ compared to the summer (see fig. 3.5 a and b). This would indicate animals acclimatised to higher summer temperatures in the field retained this ability irrespective of acclimation temperature in the laboratory during the experiments. The highest temperature at which the animals ‘stop swimming’ was achieved with the acclimation temperature of 20°C and 35 ppt and the lowest at 10°C and 2 ppt (see fig. 3.5 a and b). The analysis was run again including the life stage data. Sex did not appear to significantly impact the CTMax of *G. marinus* (see table 3.5).

Table 3.4: Results of the ANOVA GLM for *G. marinus* CTMax for the endpoint of ‘stop swimming’

Factor	DF	F	P
Mass	1	3.88	0.051
Season	1	71.95	<0.001
Salinity	2	18.21	<0.001
Temperature	1	51.56	<0.001
Season * Salinity	2	0.37	0.690
Season* temp	1	31.40	<0.001
Salinity * temp	2	9.86	<0.001
Season*salinity*temp	2	3.18	0.045
Error	114		

3.3. RESULTS

Table 3.5: Results of the ANOVA GLM for *G. marinus* CTMax for the endpoint of 'stop swimming' including life stage

Factor	DF	F	P
Mass	1	7.67	0.007
Season	1	112.17	<0.001
Salinity	2	21.09	<0.001
Temperature	1	53.28	<0.001
Life stage	1	1.64	0.203
Season * Salinity	2	0.11	0.898
Season * temp	1	34.84	<0.001
Season*life stage	1	2.55	0.114
Salinity * temp	2	16.63	<0.001
Salinity*life stage	2	0.76	0.471
Temp*life stage	1	1.41	0.238
Season*salinity*temp	2	1.77	0.176
Season*salinity*life stage	2	2.25	0.111
Season*temp*life stage	1	0.90	0.345
Salinity*temp*life stage	2	2.74	0.070
Season*salinity*temp*life stage	2	0.44	0.645
Error	99		

3.3. RESULTS

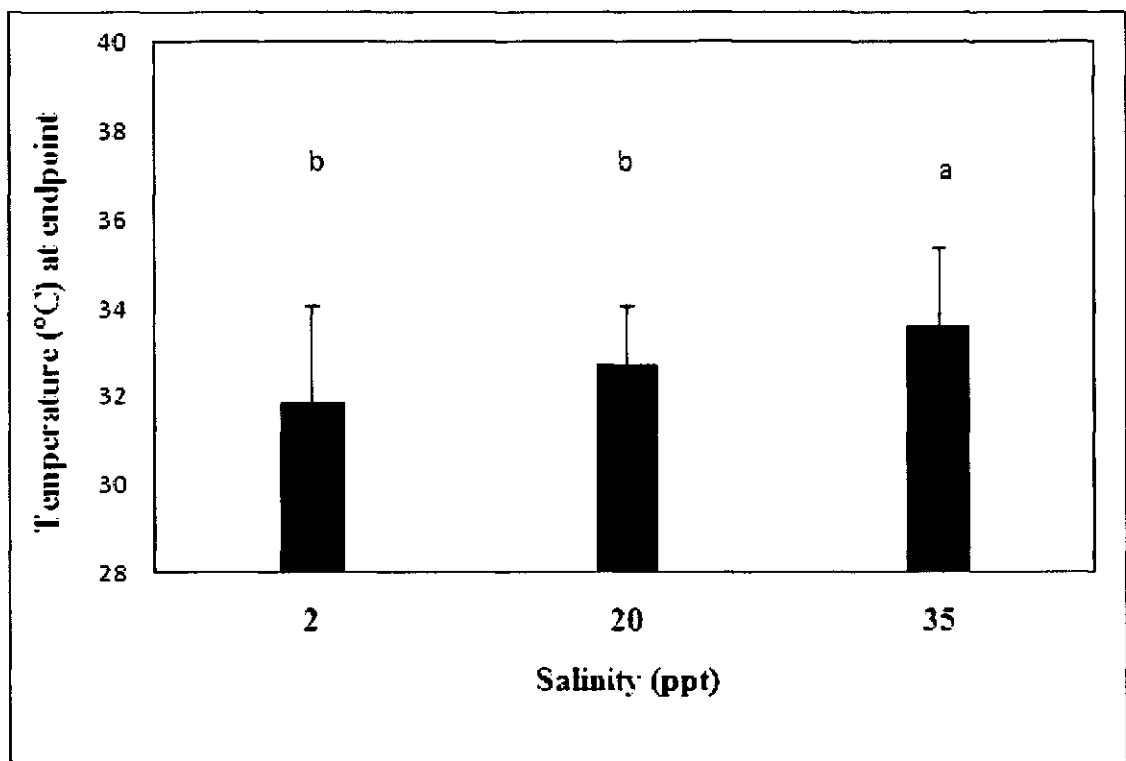


Figure 3.4: Average CTMax *G. marinus* temperature (°C) at endpoint of 'stop swimming' with acclimation temperatures (10 and 20°C) and season pooled. Error bars are standard deviations, letters are pairwise comparisons, number of data points (n) ranged between 35 and 40.

3.3. RESULTS

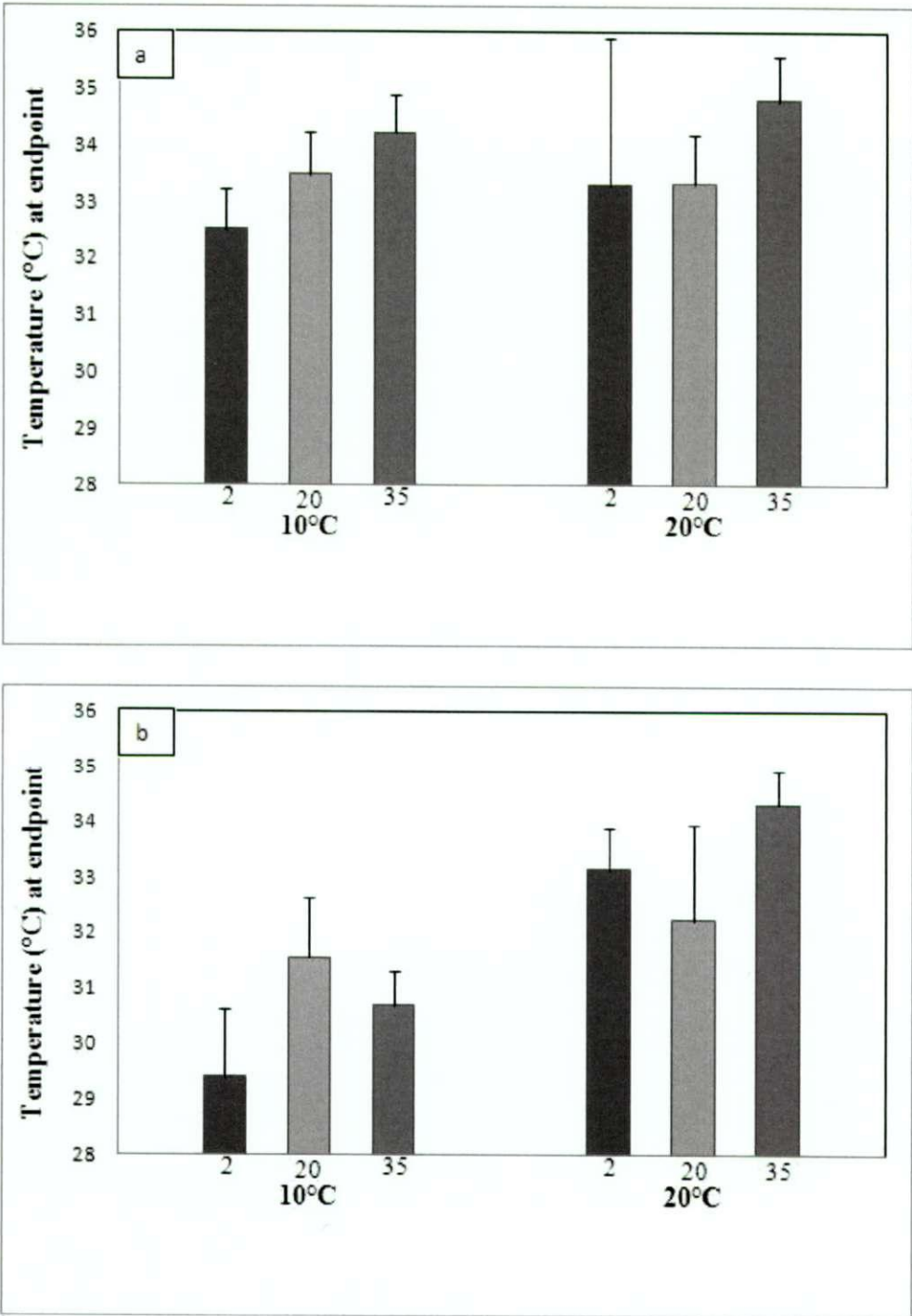


Figure 3.5: CTMax for *G. marinus* during the (a) summer and (b) winter treatments, temperature (°C) at endpoint of 'stop swimming' for each of the two acclimation temperatures (10 and 20°C) and each of the three salinities (2, 20 and 35 ppt). Error bars are standard deviations, number of data points (n) ranged between 7 and 11.

3.3. RESULTS

G. chevreuxi Salinity and temperature were both seen to significantly effect the upper thermal limit of *G. chevreuxi*. The endpoint temperature at 35 ppt was significantly different to 2 ($P = 0.011$) and 20 ppt ($P < 0.001$) (see fig. 3.6). Animals during the summer were found to have significantly different temperatures at which they 'stop swimming' with respect to the acclimation temperature (see table 3.6). The same trend was also seen during the winter (see fig. 3.7 a and b). This indicates acclimation to the test temperature overcoming the effects of seasonality. The analysis was run again including life stages and no significant result was found (see table 3.7).

Table 3.6: Results of the ANOVA GLM for *G. chevreuxi* CTMax for the endpoint of 'stop swimming'

Factor	DF	F	P
Mass	1	11.25	0.001
Season	1	3.86	0.052
Salinity	2	9.06	<0.001
Temperature	1	211.52	<0.001
Season * Salinity	2	10.54	<0.001
Season* temp	1	23.55	<0.001
Salinity * temp	2	2.27	0.108
Season*salinity*temp	2	7.09	0.001
Error	124		

3.3. RESULTS

Table 3.7: Results of the GLM for *G. chevreuxi* CTMax for the endpoint of 'stop swimming' including life stage

Factor	DF	F	P
Mass	1	5.86	0.017
Season	1	2.39	0.125
Salinity	2	7.24	0.001
Temperature	1	165.24	<0.001
Life stage	1	0.06	0.808
Season * Salinity	2	8.49	<0.001
Season* temp	1	19.79	<0.001
Season*life stage	1	2.43	0.122
Salinity * temp	2	1.54	0.219
Salinity*life stage	2	1.61	0.205
Temp*life stage	1	0.10	0.754
Season*salinity*temp	2	4.87	0.009
Season*salinity*life stage	2	1.71	0.185
Season*temp*life stage	1	1.15	0.287
Salinity*temp*life stage	2	0.01	0.987
Season*salinity*temp*life stage	2	0.02	0.976
Error	110		

3.3. RESULTS

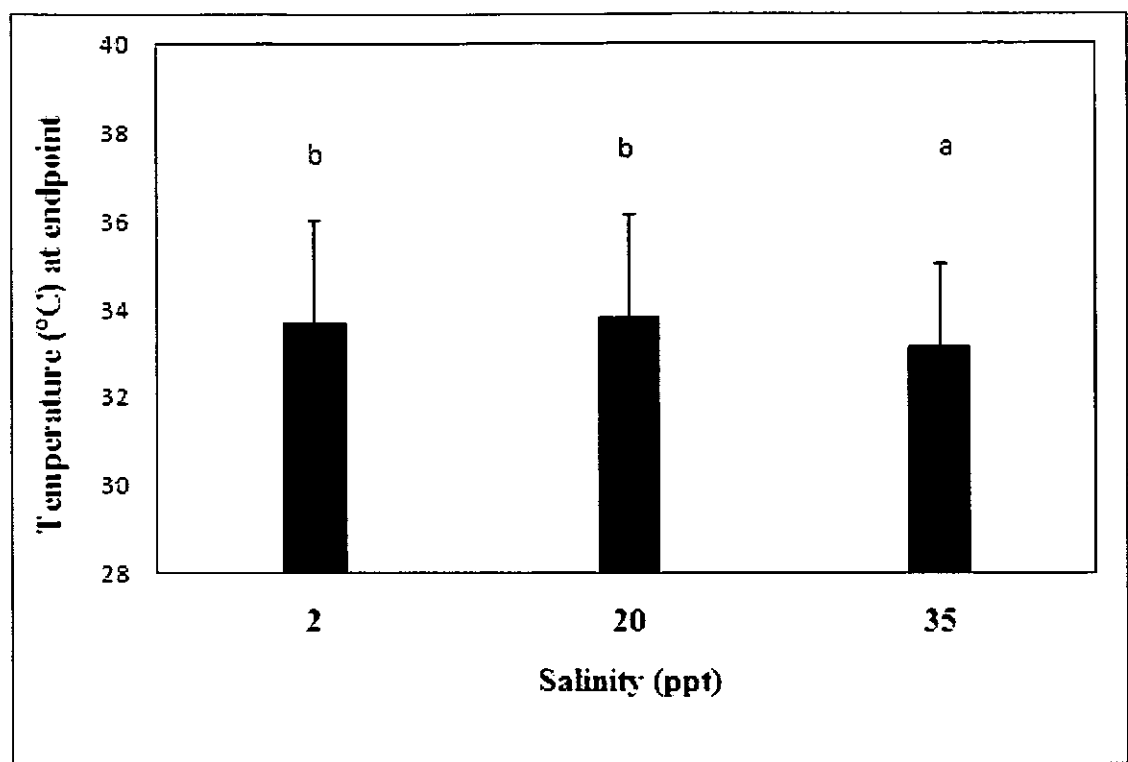


Figure 3.6: Average CTMax *G. chevreuxi* temperature (°C) at endpoint of 'stop swimming' with acclimation temperatures (10 and 20°C) and season pooled. Error bars are standard deviations, letters are pairwise comparisons, number of data points (n) ranged between 39 and 44.

3.3. RESULTS

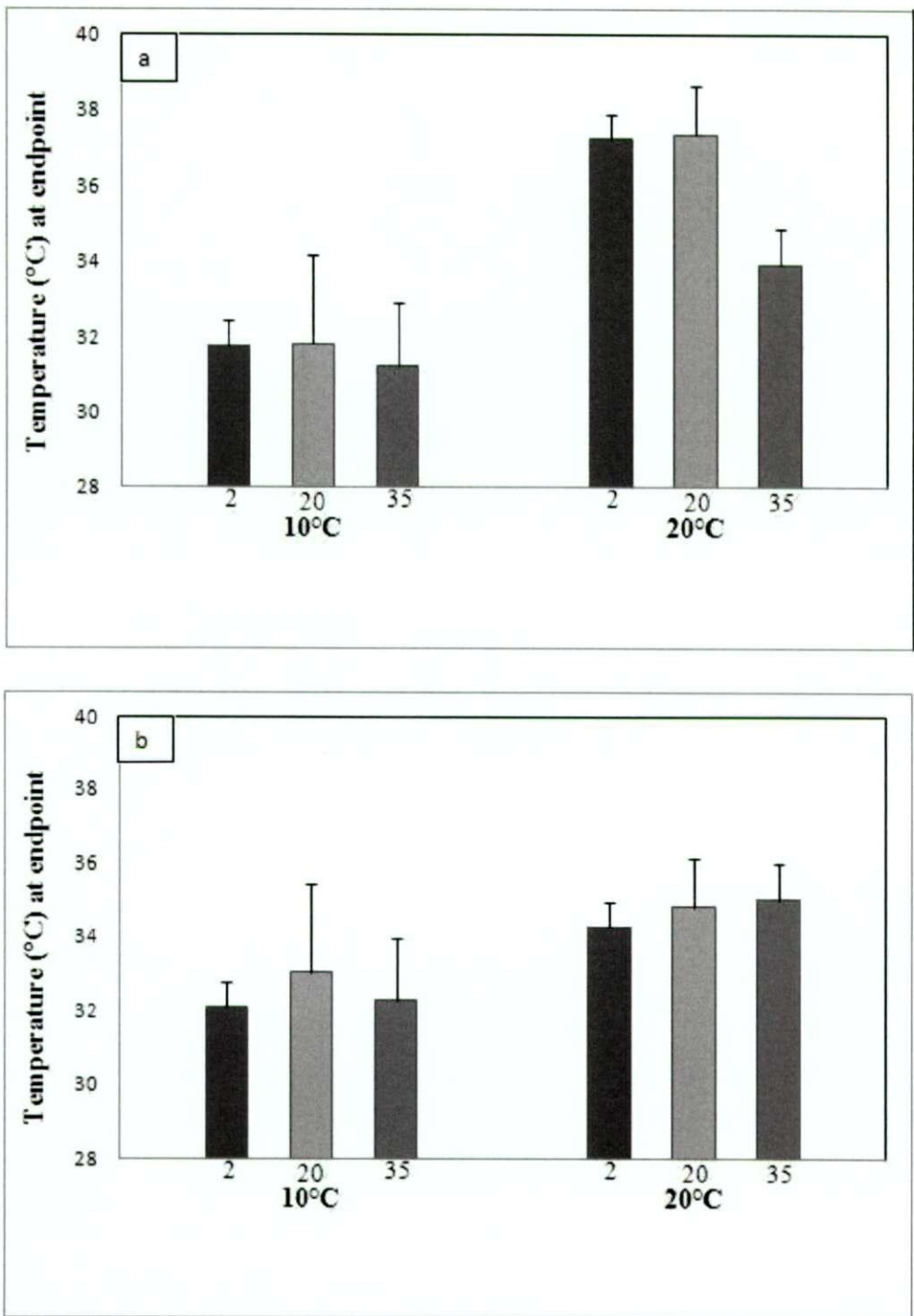


Figure 3.7: CTMax for *G. chevreuxi* during the (a) summer and (b) winter treatments, temperature (°C) at endpoint of 'stop swimming' for each of the two acclimation temperatures (10 and 20°C) and each of the three salinities (2, 20 and 35 ppt). Error bars are standard deviations, number of data points (n) ranged between 5 and 12.

3.3. RESULTS

3.3.2 CTMin

Ecologically relevant endpoint: ‘no movement when prodded’ The lower thermal tolerance of all three species was found to differ significantly with all factors (see table 3.8), and this was further confirmed by the pairwise analysis which showed that all species ($P < 0.001$) differed significantly and that the endpoint temperature at 20 ppt differed significantly from 2 ($P = 0.002$) and 35 ppt ($P < 0.001$). When the GLM was run without the *C. volutator* data, the temperature at the endpoint differed significantly for all factors except salinity (see table 3.8). On the basis of these tests and in order to not complicate the discussion of the results, it was decided that each amphipod species should be treated separately.

Endpoint of death: ‘no movement when warmed’ The endpoint of death was effected by the same factors as ‘no movement when prodded’ with one notable exception (see table 3.8). It was found that the effect of season was lost on the endpoint of death. The pairwise comparisons showed the same significant difference between endpoint temperatures between species ($P < 0.001$); the salinity of 20 ppt differed significantly from 2 ($P = 0.008$) and 35 ppt ($P < 0.001$). No further analysis of this data was undertaken, as the study focused on the more ecologically relevant endpoint.

3.3. RESULTS

Table 3.8: Results of the ANOVA GLM for CTMin endpoint of 'no movement' for all three species, the results for Gammarids as well as the the endpoint of 'death'

Factor	'stop swimming'						'death'		
	All species			Gammarids			All species		
	DF	F	P	DF	F	P	DF	F	P
Mass	1	61.58	<0.001	1	57.04	<0.001	1	26.12	<0.001
Species	2	266.45	<0.001	1	83.86	<0.001	2	186.12	<0.001
Season	1	18.03	<0.001	1	39.39	<0.001	1	3.25	0.072
Salinity	2	12.55	<0.001	2	1.54	0.216	2	12.98	<0.001
Temperature	1	24.73	<0.001	1	5.57	0.019	1	7.46	0.007
Species*season	2	23.22	<0.001	1	20.98	0.003	2	20.26	<0.001
Species*salinity	4	7.43	<0.001	2	5.89	0.001	4	6.69	<0.001
Species*temp	2	4.26	0.015	1	0.03	0.868	2	12.01	<0.001
Season * Salinity	2	10.28	<0.001	2	5.97	0.003	2	6.55	0.002
Season* temp	1	11.47	0.001	1	0.79	0.376	1	1.04	0.308
Salinity * temp	2	27.93	<0.001	2	16.15	<0.001	2	10.39	<0.001
Season*salinity*temp	2	1.85	0.159	2	3.11	0.047	2	0.48	0.619
Species*season*salinity	4	13.51	<0.001	2	9.04	<0.001	4	4.85	0.001
Species*season*temp	2	5.69	0.004	1	0.28	0.595	2	1.78	0.170
Species*salinity*temp	4	3.42	0.009	2	5.55	0.004	4	0.94	0.439
Species*season*salinity*temp	4	16.03	<0.001	2	14.56	<0.001	4	8.13	<0.001
Error	346			223			346		

C. volutator The lower thermal tolerance of *C. volutator* was significantly effected by all factors except season (see table 3.9). The pairwise comparisons showed all salinities to have significantly difference endpoint temperatures ($P<0.05$) (see fig. 3.8). There was an interaction between salinity and temperature. During the winter, acclimation temperature affects the CTMin, whereas salinity appears to be more important during the summer (see fig. 3.9 a and b). When the life stage was incorporated into the analysis, no new significant results were detected (see table 3.10).

3.3. RESULTS

Table 3.9: Results of the ANOVA GLM for *C. volutator* CTMin for the endpoint of 'no movement'

Factor	DF	F	P
Mass	1	39.95	<0.001
Season	1	3.64	0.059
Salinity	2	36.84	<0.001
Temperature	1	38.49	<0.001
Season * Salinity	2	40.60	<0.001
Season* temp	1	34.09	<0.001
Salinity * temp	2	16.40	<0.001
Season*salinity*temp	2	19.59	<0.001
Error	121		

Table 3.10: Results of the ANOVA GLM for *C. volutator* CTMin for the endpoint of 'no movement' including life stage

Factor	DF	F	P
Mass	1	2.67	0.105
Salinity	2	4.80	0.010
Temperature	1	18.18	<0.001
Life stage	2	1.82	0.167
Salinity * temp	2	6.74	0.002
Salinity*life stage	4	1.63	0.170
Temp*life stage	2	0.93	0.396
Salinity*temp*life stage	4	1.83	0.128
Error	115		

3.3. RESULTS

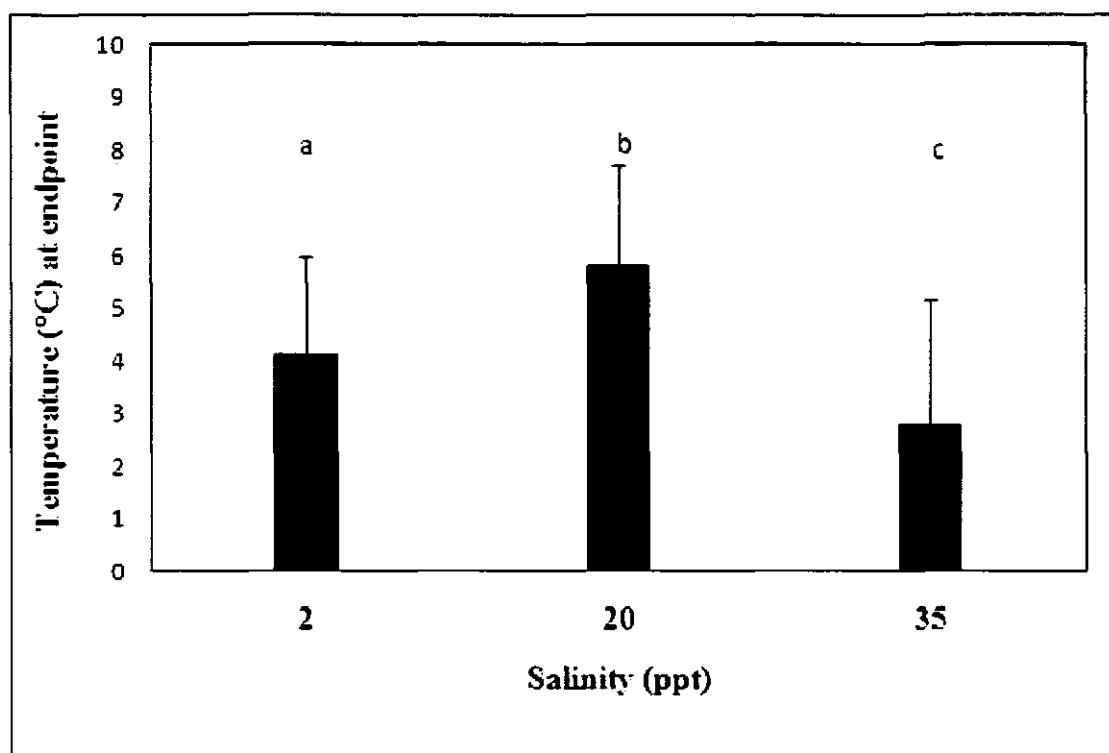


Figure 3.8: Average CTMin *C. volutator* temperature (°C) at endpoint of 'no movement' with acclimation temperatures (10 and 20°C) and season pooled. Error bars are standard deviations, letters are pairwise comparisons, number of data points (n) ranged between 35 and 44.

3.3. RESULTS

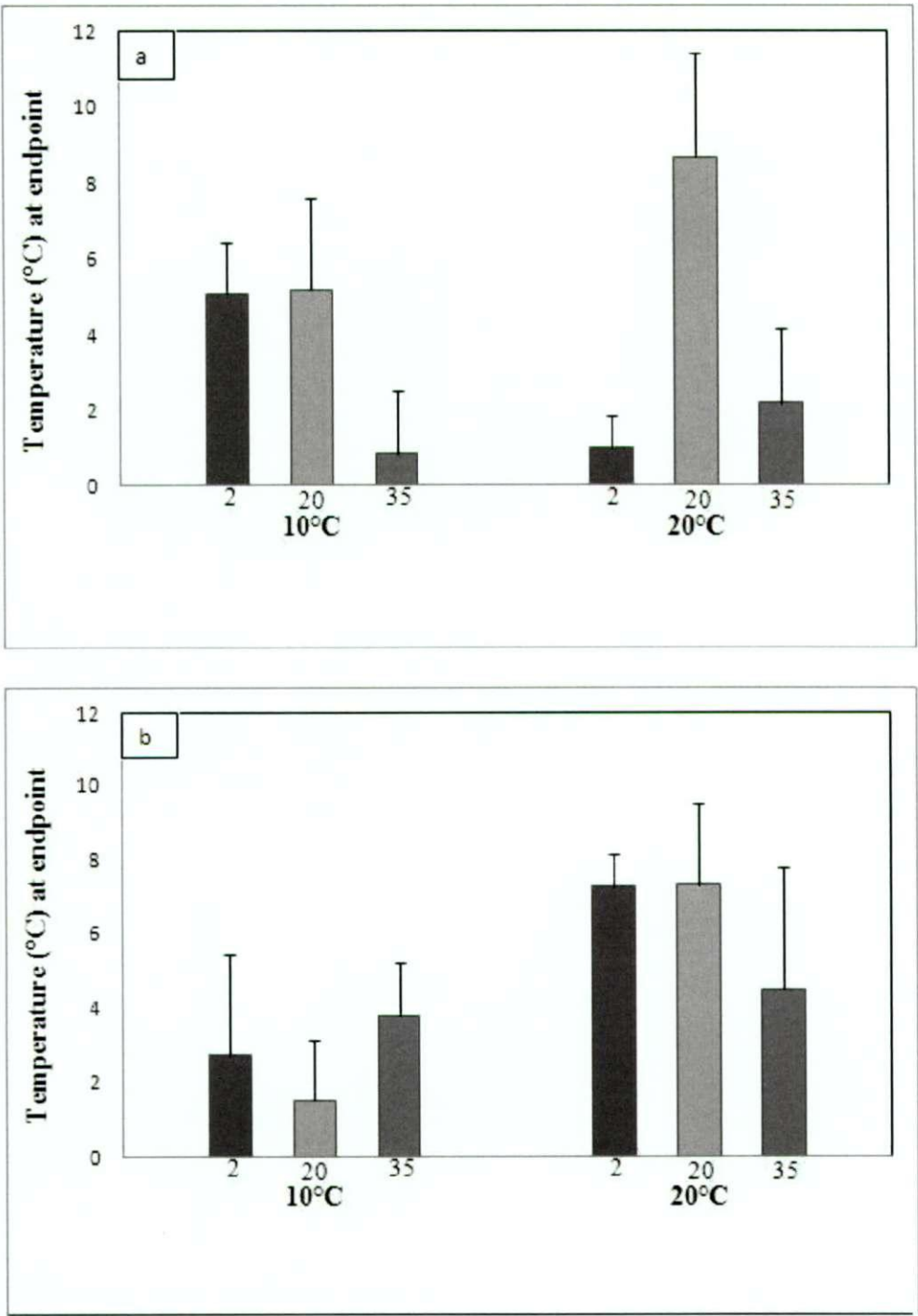


Figure 3.9: CTMin for *C. volutator* during the (a) summer and (b) winter treatments, temperature (°C) at endpoint of 'no movement' for each of the two acclimation temperatures (10 and 20°C) and each of the three salinities (2, 20 and 35 ppt). Error bars are standard deviations, number of data points (n) ranged between 5 and 15.

3.3. RESULTS

G. marinus The lower thermal limit of *G. marinus* was significantly effected by season, with temperature and salinity only being significant when combined (see table 3.11). The pairwise test between salinities showed no significant results (see fig. 3.10). Animals collected during the winter were found to have a significantly lower CTMin when compared the individuals collected during the summer (see figs. 3.11 a and b and 3.12). Animals did not acclimate to the laboratory temperatures and retained their natural CTMin. The interaction between acclimation temperature and salinity appears to be much more complex and could possibly be a spurious result of the statistical analysis. When the analysis was re-run including the life stage of the individual, no new significant differences were detected (see table 3.12).

Table 3.11: Results of the ANOVA GLM for *G. marinus* CTMin for the endpoint of 'no movement'

Factor	DF	F	P
Mass	1	53.60	<0.001
Season	1	56.54	<0.001
Salinity	2	0.74	0.481
Temperature	1	3.02	0.085
Season * Salinity	2	0.60	0.552
Season* temp	1	0.97	0.326
Salinity * temp	2	6.12	0.003
Season*salinity*temp	2	2.17	0.119
Error	102		

3.3. RESULTS

Table 3.12: Results of the ANOVA GLM for *G. marinus* CTMin for the endpoint of 'no movement' including life stage

Factor	DF	F	P
Mass	1	30.79	<0.001
Season	1	51.52	<0.001
Salinity	2	1.34	0.267
Temperature	1	2.92	0.091
Life stage	1	1.05	0.309
Season * Salinity	2	1.07	0.347
Season* temp	1	0.14	0.705
Season*life stage	1	3.61	0.061
Salinity * temp	2	6.04	0.003
Salinity*life stage	2	1.28	0.284
Temp*life stage	1	0.07	0.788
Season*salinity*temp	2	3.30	0.041
Season*salinity*life stage	2	0.18	0.832
Season*temp*life stage	1	3.43	0.068
Salinity*temp*life stage	2	1.25	0.291
Season*salinity*temp*life stage	2	1.62	0.203
Error	89		

3.3. RESULTS

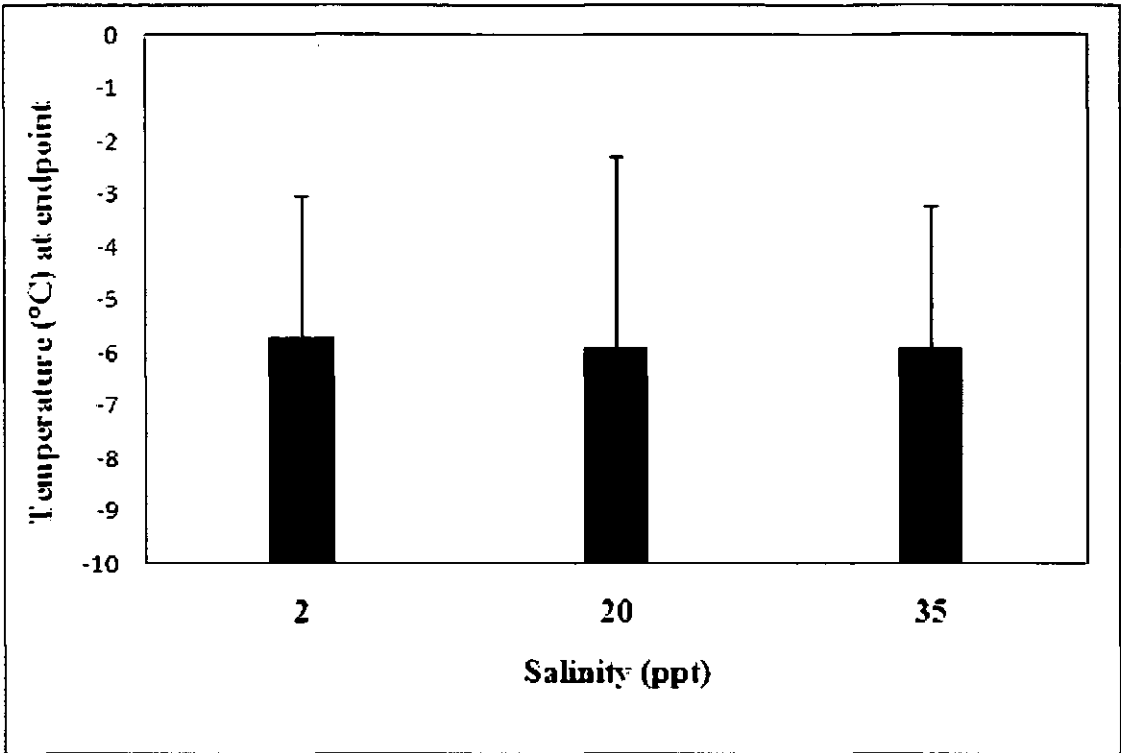


Figure 3.10: Average CTMin *G. marinus* temperature (°C) at endpoint of 'no movement' with acclimation temperatures (10 and 20°C) and season pooled. Error bars are standard deviations, number of data points (n) ranged between 30 and 38.

3.3. RESULTS

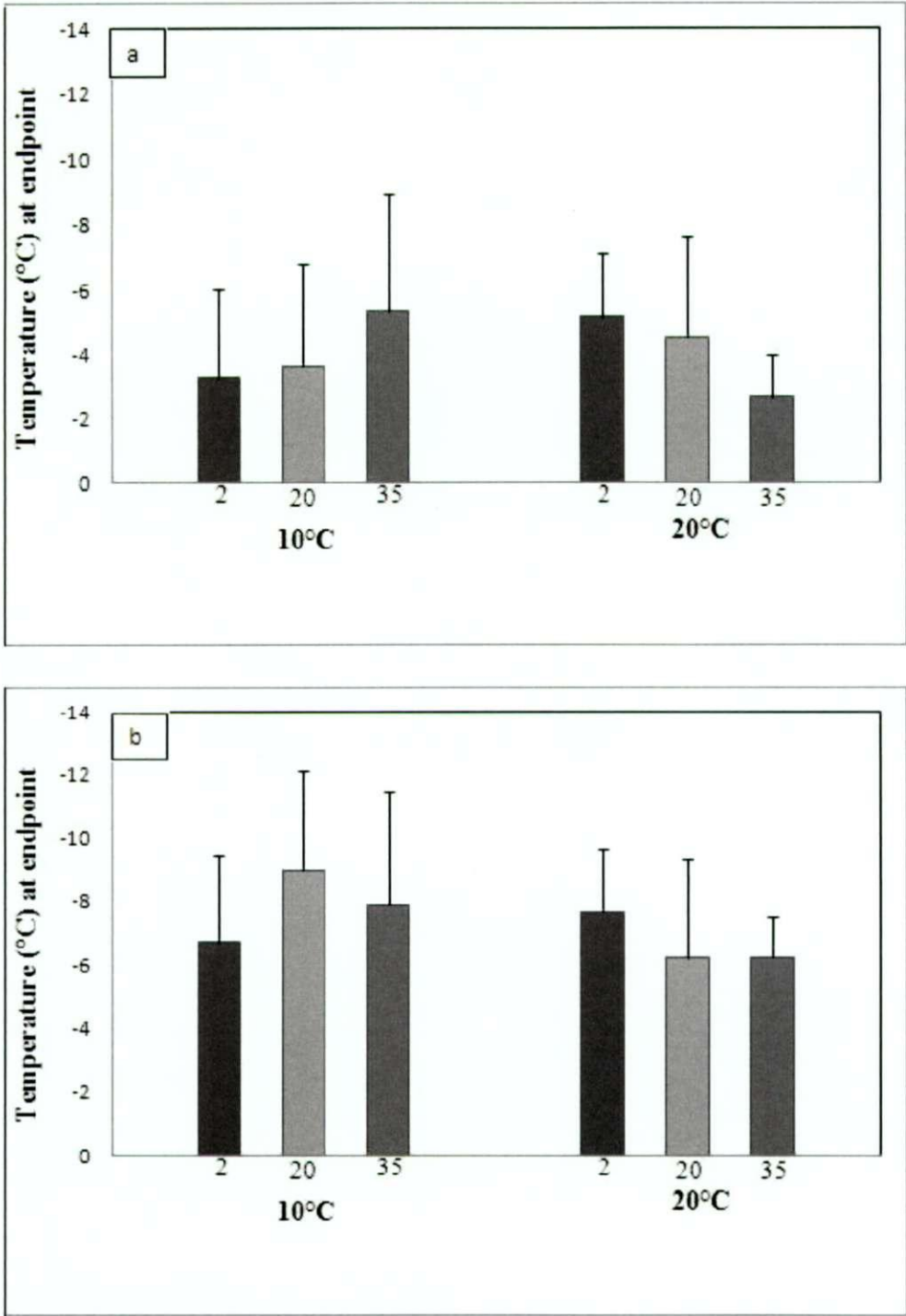


Figure 3.11: CTMin for *G. marinus* during the (a) summer and (b) winter treatments, temperature (°C) at endpoint of 'no movement' for each of the two acclimation temperatures (10 and 20°C) and each of the three salinities (2, 20 and 35 ppt). Error bars are standard deviations, number of data points (n) ranged between 5 and 11.

3.3. RESULTS

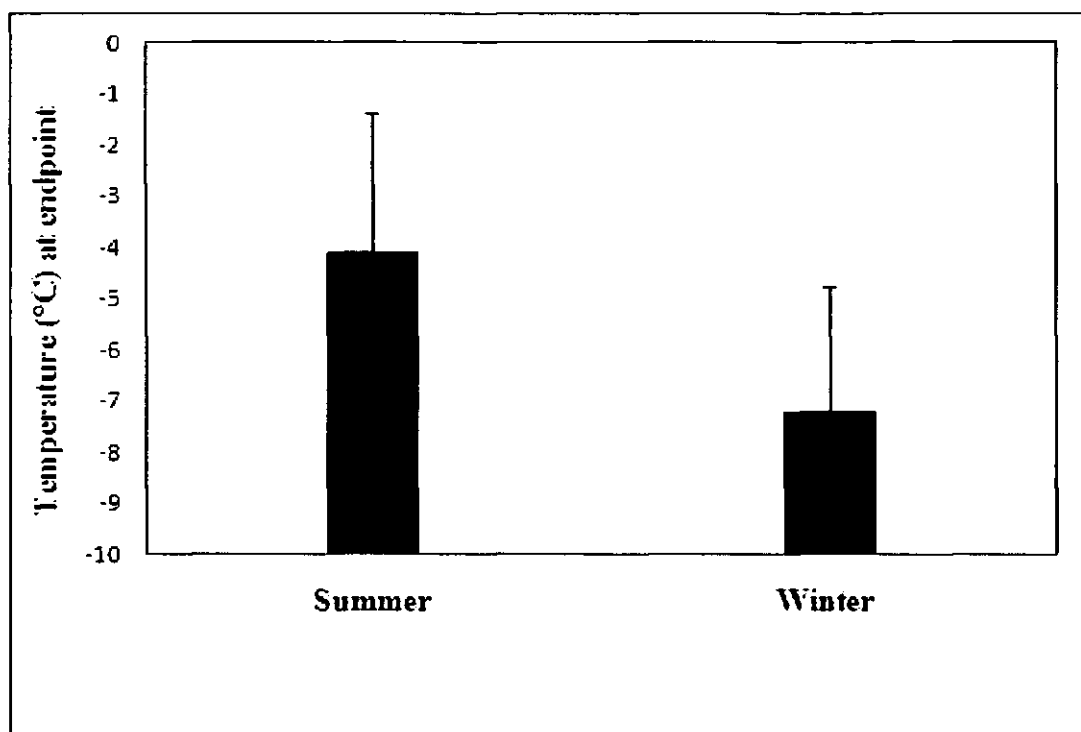


Figure 3.12: CTMin for *G. marinus* temperature (°C) at endpoint of 'no movement' with acclimation temperature and salinity treatments pooled. Error bars are standard deviations, summer $n = 45$ and winter $n = 58$.

3.3. RESULTS

G. chevreuxi Salinity was found to have a significant effect on the lower thermal limit of *G. chevreuxi*. Salinity appeared to be driving the significant results with the other factors (see table 3.13). The endpoint temperature of 20 ppt was found to differ significantly with 2 (P = 0.003) and 35 ppt (P = 0.007) in the pairwise comparisons (see fig. 3.13). The CTMin appeared to be similar regardless of the acclimation temperature (see fig. 3.14). The lowest CTMin was found to be at 20 ppt during the summer and 35 ppt during the winter (see fig. 3.14 a and b). When life stage was included in the analysis, new significant results were found (see table 3.14). Males appeared to have a consistent CTMin irrespective of the salinity and acclimation temperature (see fig. 3.15a). Females had a much more variable range of CTMin with the lowest being found at 20 ppt and 10°C and 2 ppt at 20°C (see fig. 3.15b). Overall, males had a lower CTMin compared to females (see fig. 3.16).

Table 3.13: Results of the ANOVA GLM for *G. chevreuxi* CTMin for the endpoint of ‘no movement’

Factor	DF	F	P
Mass	1	4.92	0.028
Season	1	1.45	0.231
Salinity	2	6.86	0.002
Temperature	1	2.29	0.133
Season * Salinity	2	15.89	<0.001
Season* temp	1	0.05	0.827
Salinity * temp	2	15.26	<0.001
Season*salinity*temp	2	17.02	<0.001
Error	120		

3.3. RESULTS

Table 3.14: Results of the ANOVA GLM for *G. chevreuxi* CTMin for the endpoint of 'no movement' including life stages

Factor	DF	F	P
Mass	1	2.15	0.146
Season	1	3.50	0.064
Salinity	2	4.10	0.019
Temperature	1	1.71	0.193
Sex	1	11.39	0.001
Season * Salinity	2	17.91	<0.001
Season*temp	1	0.25	0.618
Season*Sex	1	0.64	0.425
Salinity * temp	2	14.80	<0.001
Salinity*sex	2	3.88	0.024
Temp*sex	1	2.13	0.147
Season*salinity*temp	2	14.20	<0.001
Season*salinity*sex	2	0.51	0.603
Season*temp*sex	1	0.19	0.664
Salinity*temp*sex	2	4.77	0.010
Season*salinity*temp*sex	2	2.90	0.060
Error	107		

3.3. RESULTS

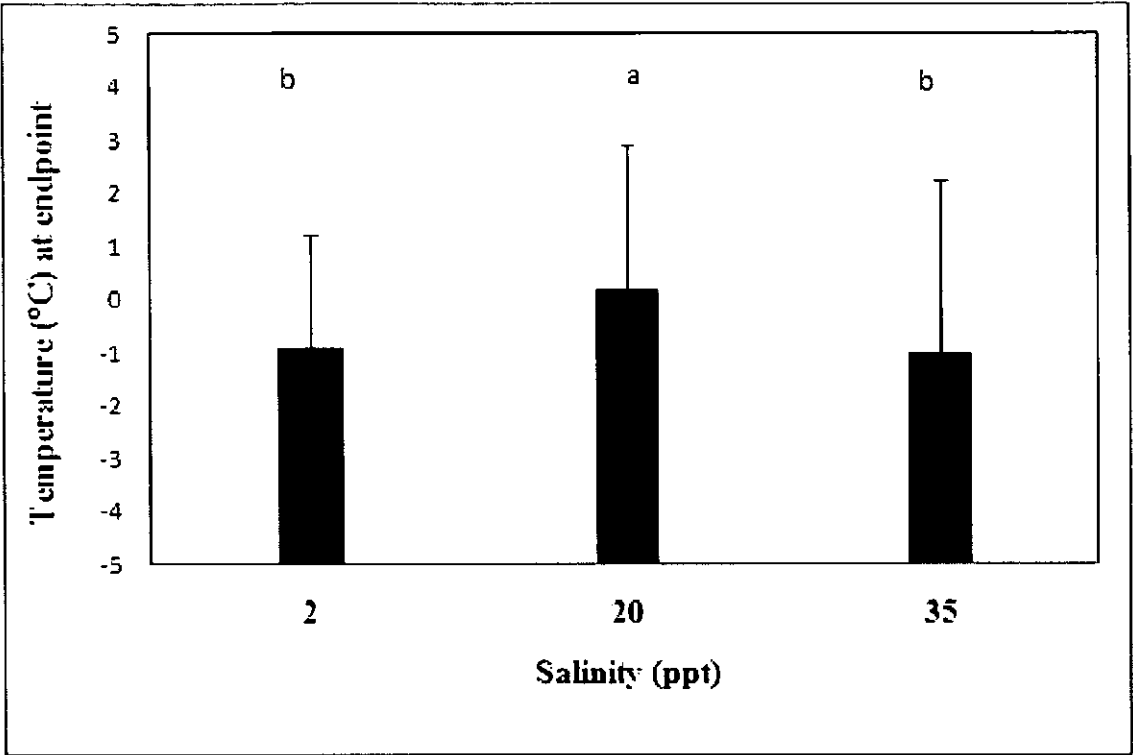


Figure 3.13: Average CTMin *G. chevreuxi* temperature (°C) at endpoint of 'no movement' with acclimation temperatures (10 and 20°C) and season pooled. Error bars are standard deviations, letters are pairwise comparisons, number of data points (n) ranged between 38 and 44.

3.3. RESULTS

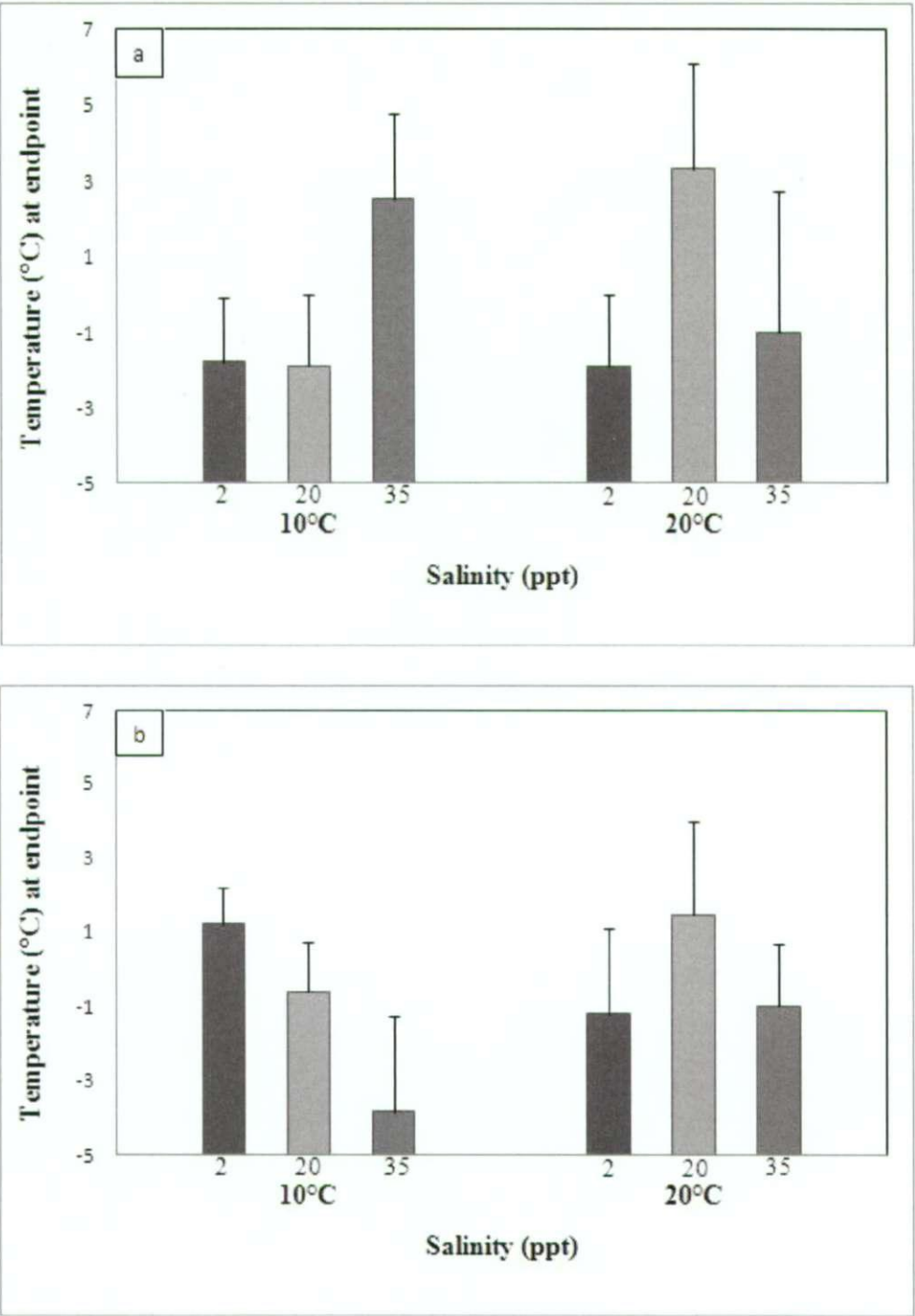


Figure 3.14: CTMin for *G. chevreuxi* during the (a) summer and (b) winter treatments, temperature (°C) at endpoint of 'no movement' for each of the two acclimation temperatures (10 and 20°C) and each of the three salinities (2, 20 and 35 ppt). Error bars are standard deviations, number of data points (n) ranged between 5 and 11.

3.3. RESULTS

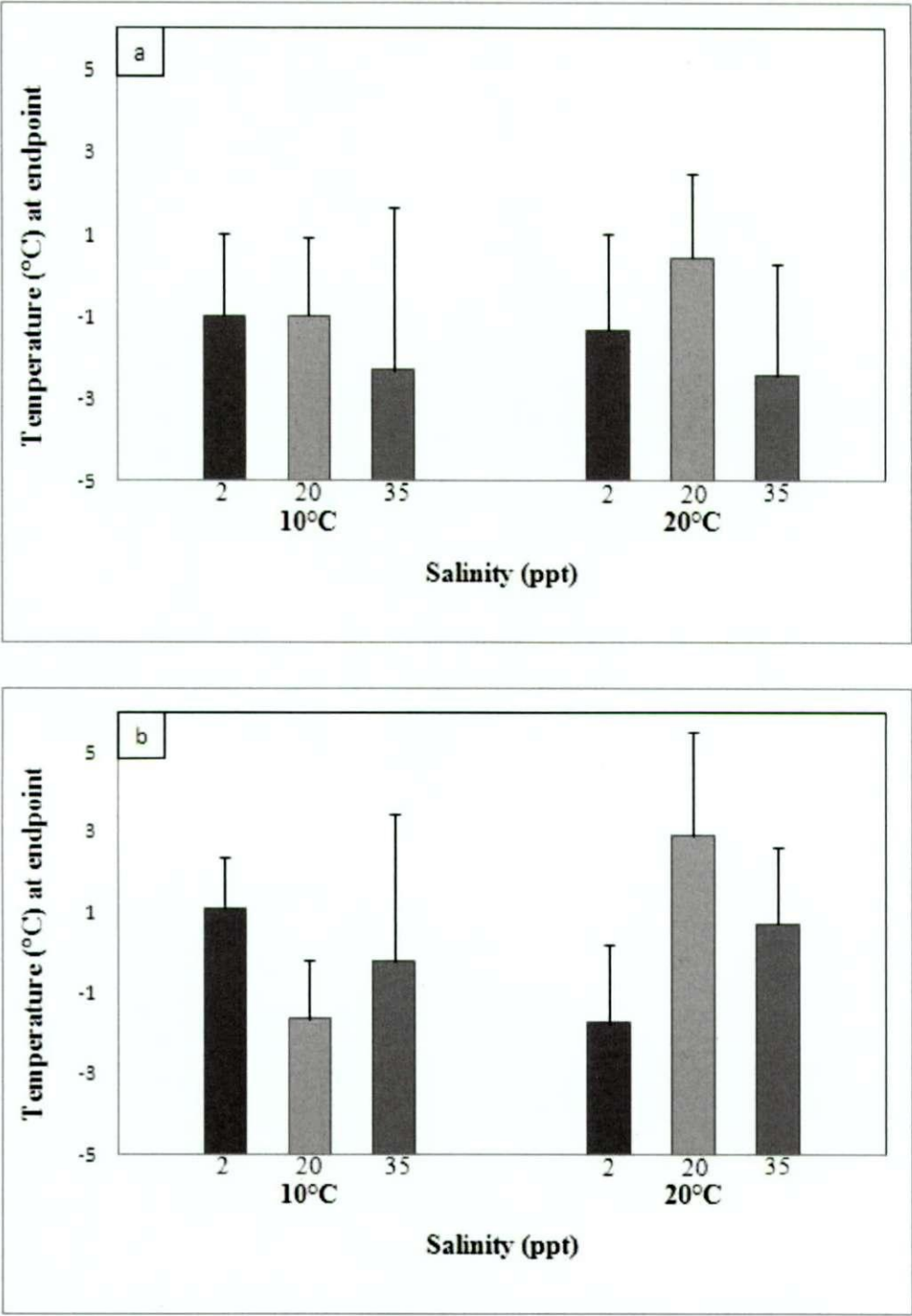


Figure 3.15: CTMin for *G. chevreuxi* for (a) males and (b) females, temperature (°C) at end-point of 'no movement' for each of the two acclimation temperatures (10 and 20°C) and each of the three salinities (2, 20 and 35ppt), (c) pooled data for sexes. Error bars are standard deviations, number of data points (n) ranged between 5 and 10.

3.3. RESULTS

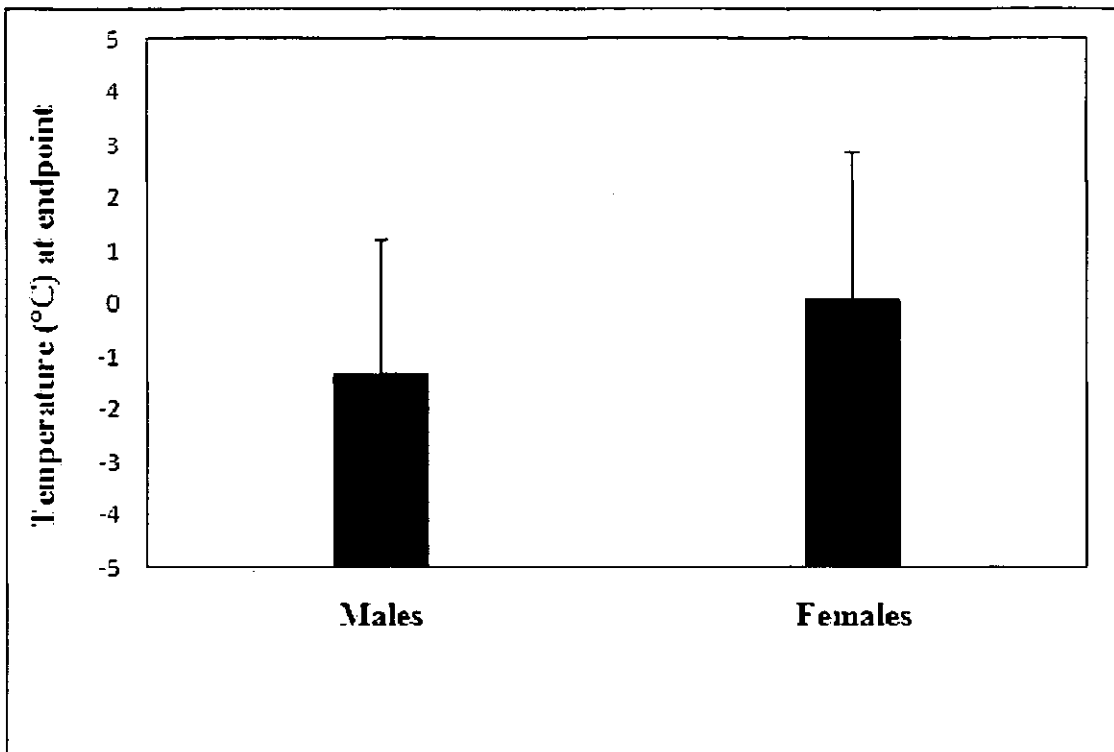


Figure 3.16: CTMin for *G. chevreuxi* temperature (°C) at endpoint of 'no movement' pooled data for sexes. Error bars are standard deviations, male $n = 50$ and female $n = 42$.

3.3.3 Temperature range

In order to better visualise the potential trends in the data, trendlines were fitted to the data for each acclimation and temperature combination, for each species. The equation was then used to make standardised data from a full range of body masses, for *C. volutator* and *G. chevreuxi* between 0.0001 to 0.015g and for *G. marinus* between 0.0001 and 0.25g. Each combination of acclimation temperature (10 and 20 °C) and salinity (2, 20 and 35 ppt) are displayed as linear trendlines for comparison of summer and winter for each of the three species. For example, the trendline labeled 10/35 displays the theoretical temperature range across body mass at 10°C and 35 ppt.

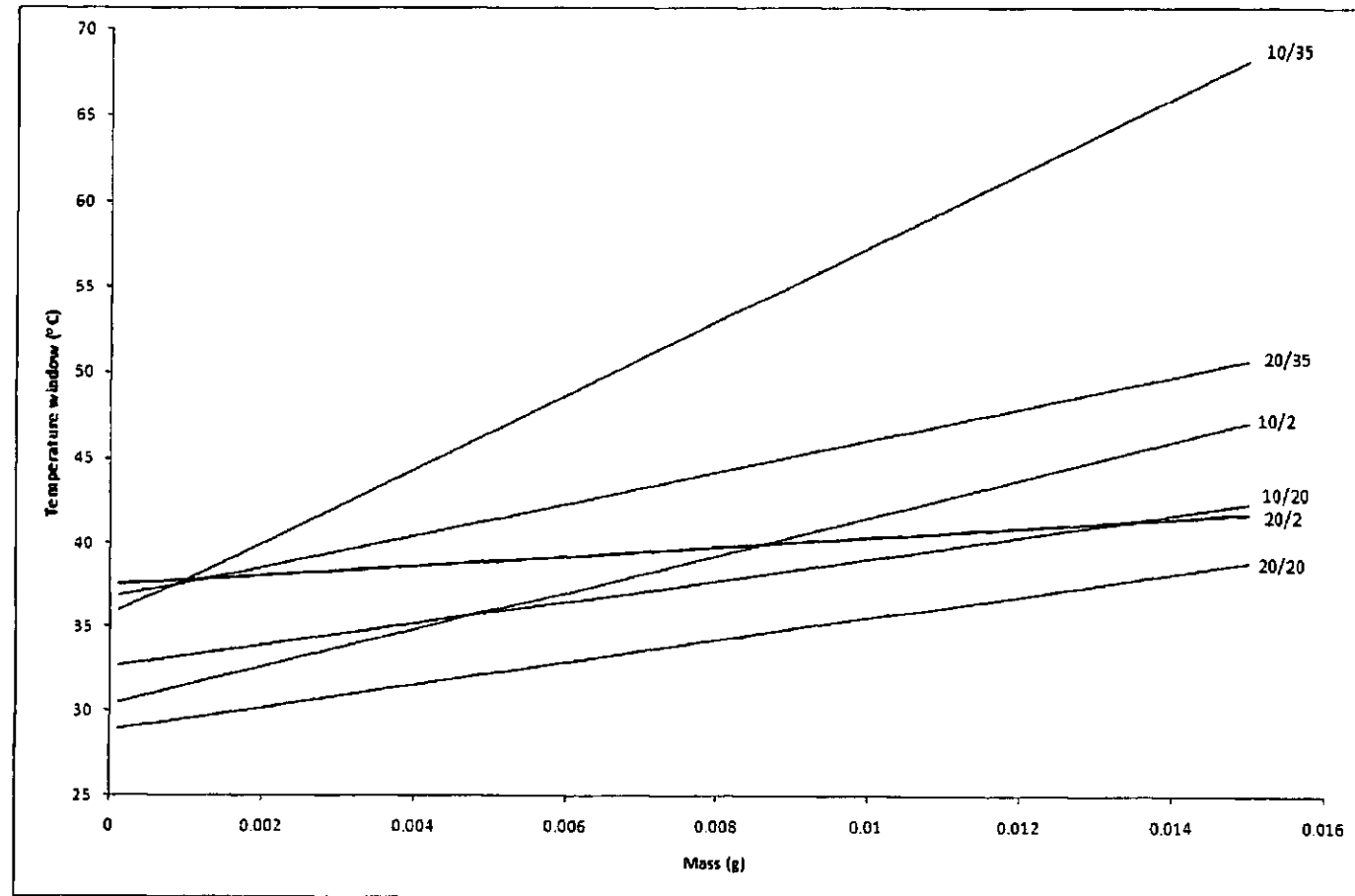


Figure 3.17: *C. volutator* trendlines (CTMax - CTMin) for the summer in temperature (°C) across a range of standardised masses (g). Trendline labels are acclimation temperature (°C)/salinity (ppt).

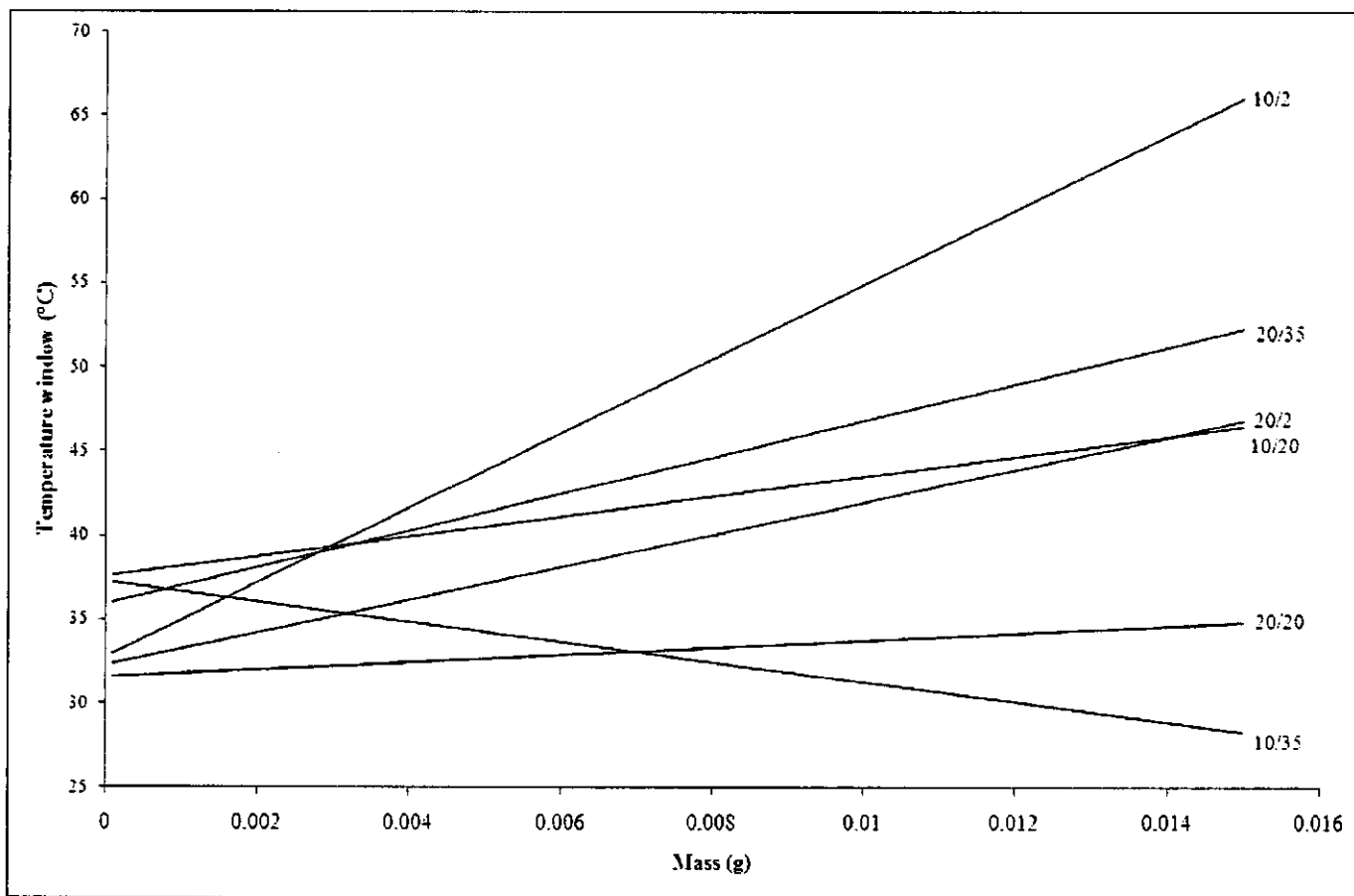


Figure 3.18: *C. volutator* trendlines (CTMax - CTMin) for the winter in temperature (°C) across a range of standardised masses (g). Trendline labels are acclimation temperature (°C)/salinity (ppt).

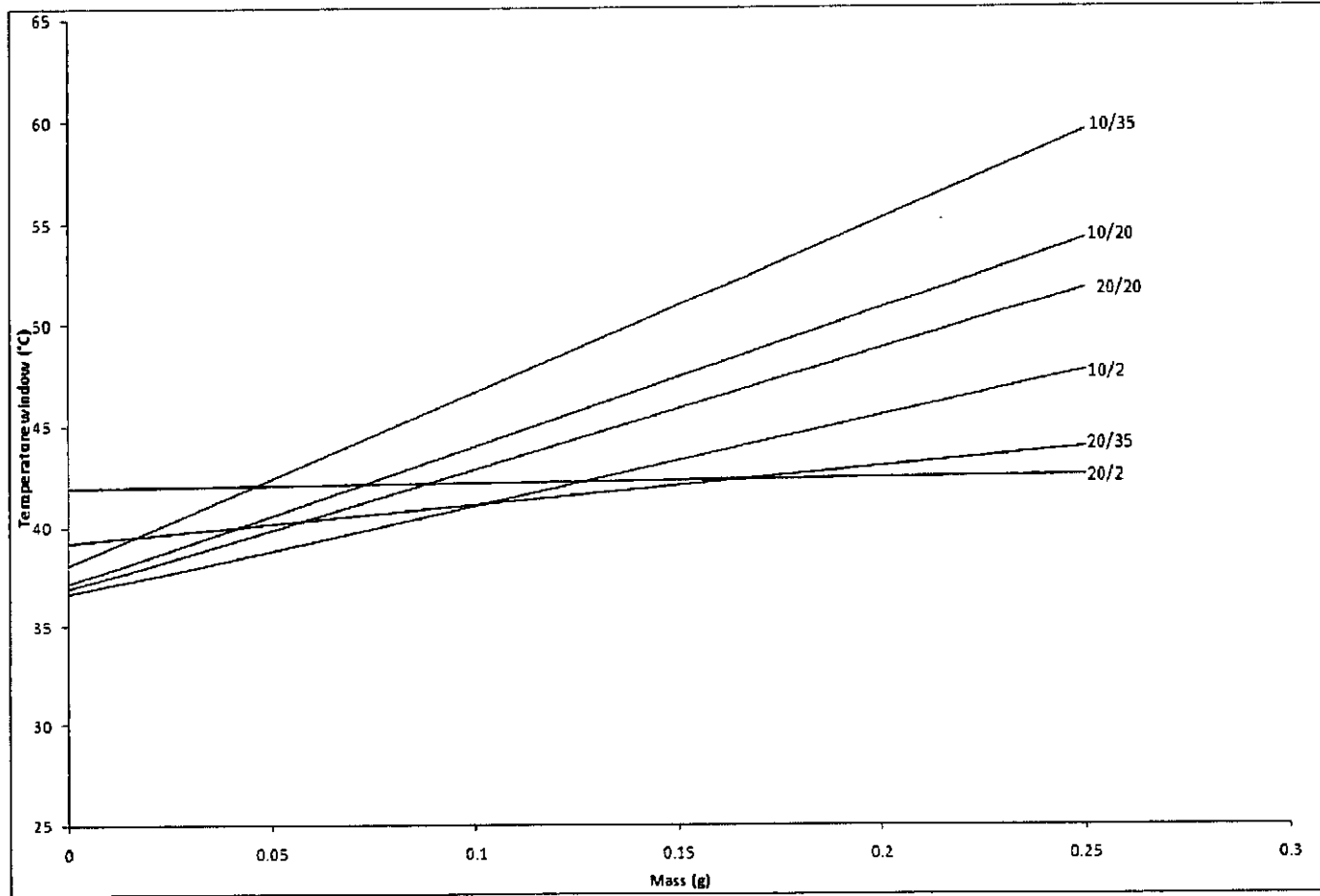


Figure 3.19: *G. marinus* trendlines (CTMax - CTMin) for the summer in temperature (°C) across a range of standardised masses (g). Trendline labels are acclimation temperature (°C)/salinity (ppt).

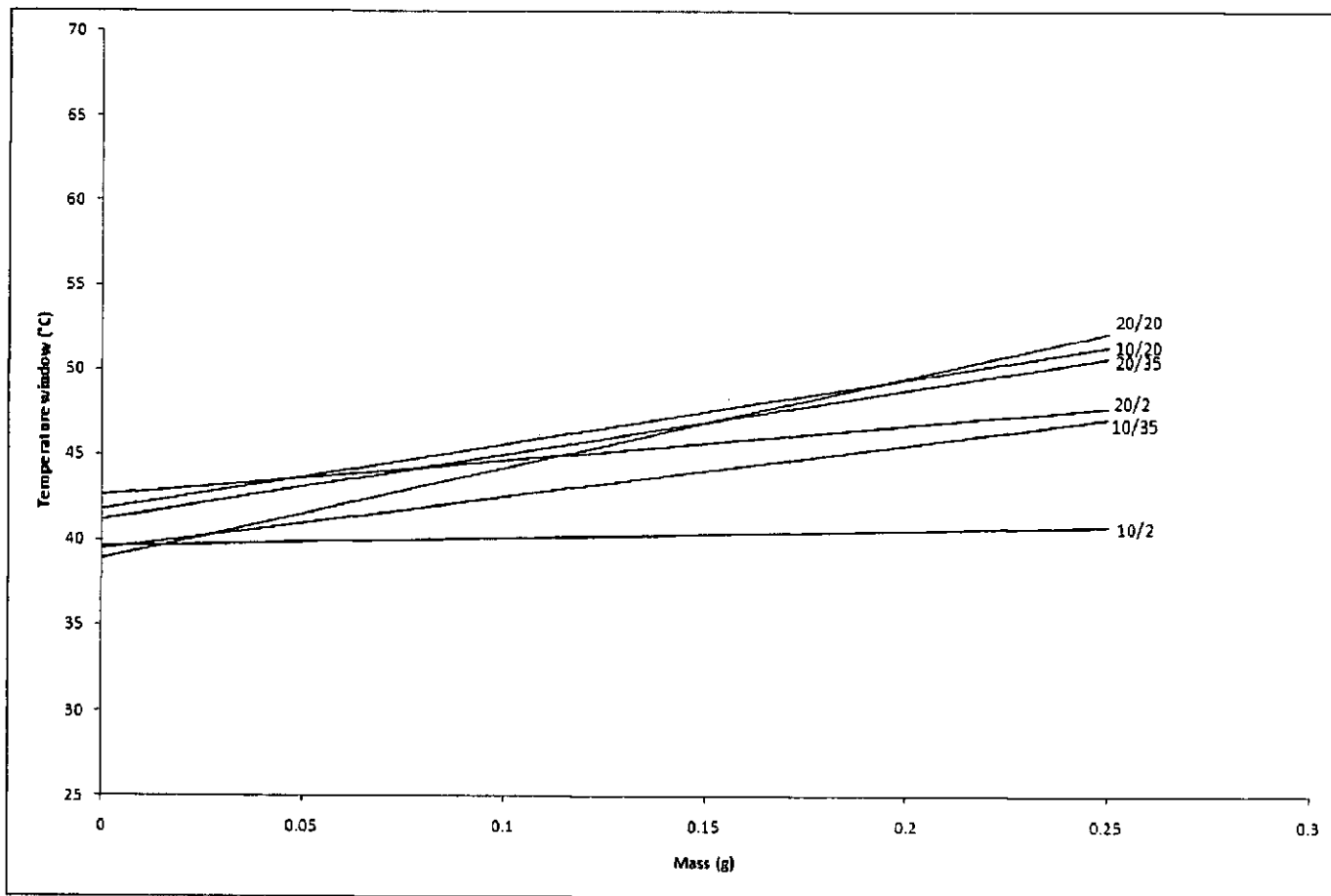


Figure 3.20: *G. marinus* trendlines (CTMax - CTMin) for the winter in temperature (°C) across a range of standardised masses (g). Trendline labels are acclimation temperature (°C)/salinity (ppt).

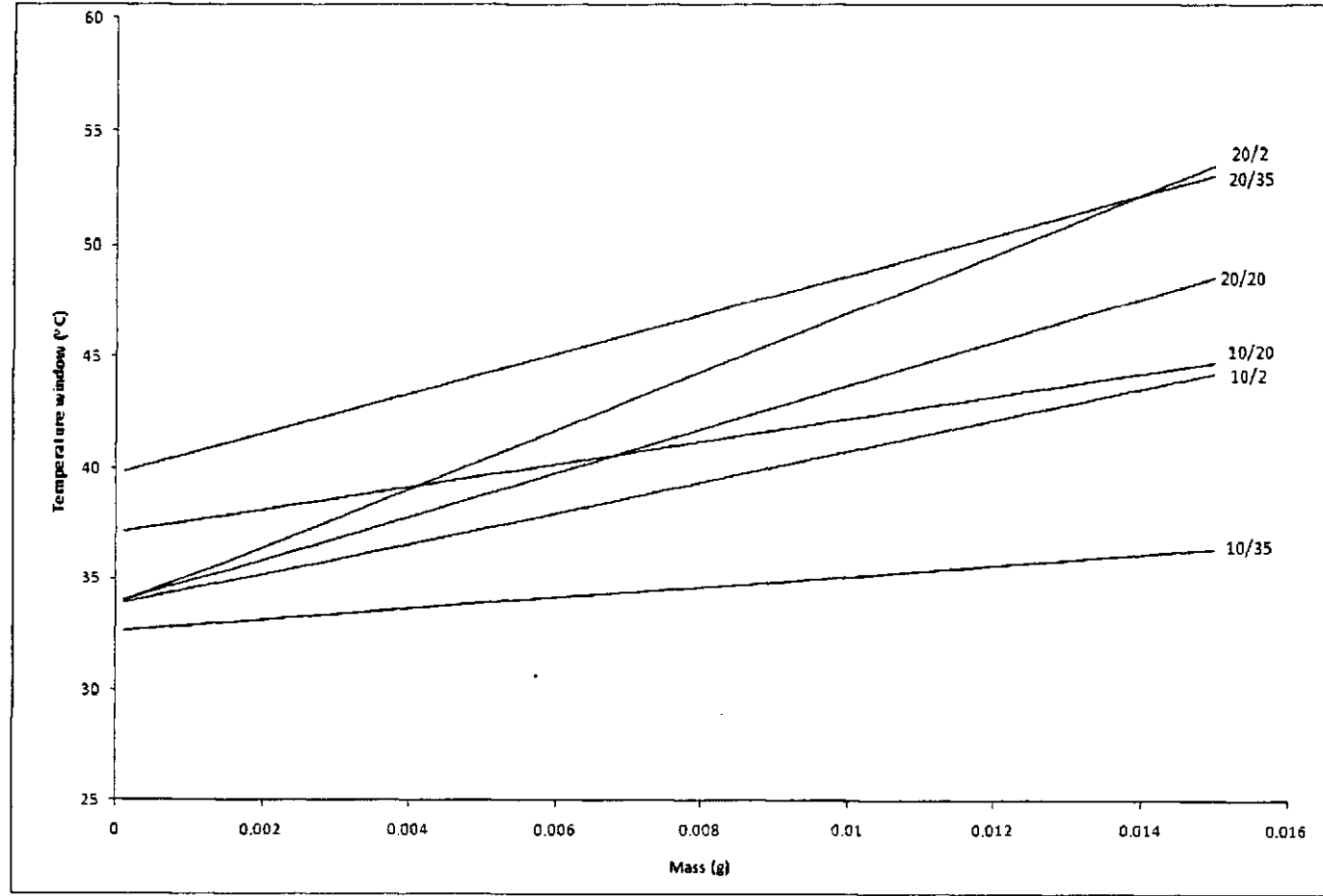


Figure 3.21: *G. chevreuxi* trendlines (CTMax - CTMin) for the summer in temperature ($^{\circ}\text{C}$) across a range of standardised masses (g). Trendline labels are acclimation temperature ($^{\circ}\text{C}$)/salinity (ppt).

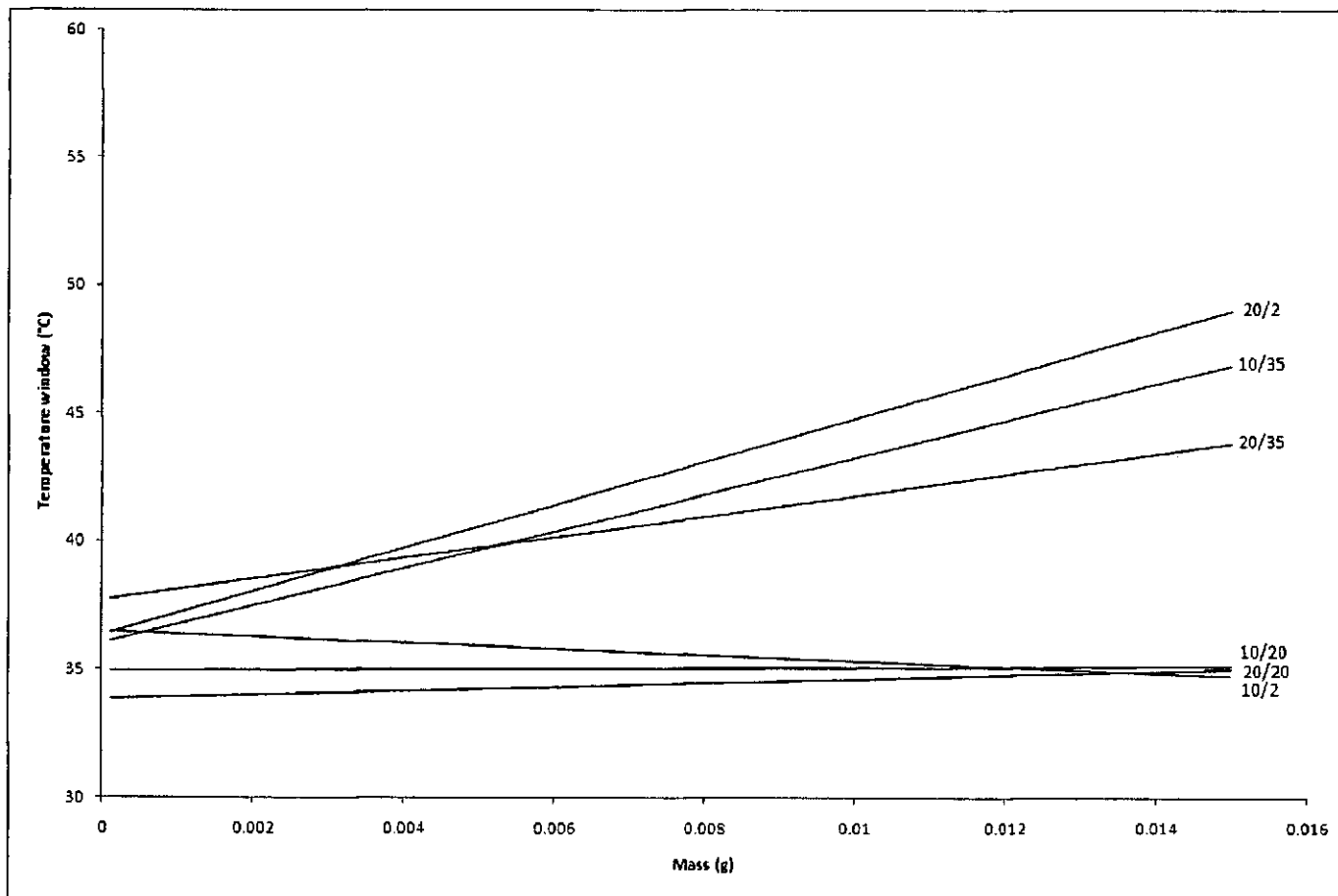


Figure 3.22: *G. chevreuxi* trendlines (CTMax - CTMin) for the winter in temperature ($^{\circ}\text{C}$) across a range of standardised masses (g). Trendline labels are acclimation temperature ($^{\circ}\text{C}$)/salinity (ppt).

3.4 Discussion

The results for the CTMax between all species were quite similar; no significant differences were found between the upper thermal tolerance of the different life stages. Acclimation to the test temperatures appeared to be occurring in all species with respect to CTMax, particularly evident in the winter. Overall, animals collected in the winter had a lower CTMin, but there is a lot of variation which might be attributed to the fact that the experiments were conducted in air. However, the lack of any significant effect of acclimation temperature on either *Gammarus* species indicates that seasonality (*G. marinus*) and salinity (*G. chevreuxi*) are much more important factors in determining CTMin, where the interaction between the factors is much more complex for *C. volutator*.

From numerous studies (Lowe & Vance 1955, Hutchison & Rowlan 1975, Lutterschmidt & Hutchison 1997a, Stillman & Somero 2000, Klok & Chown 2003, Compton et al. 2007, Sepulveda et al. 2008), it appears that there are two main choices for thermal limit experiments. One, time to death experiments, where animals are acclimated then simply dropped into extremes of hot or cold temperature and then observed until the endpoint is reached (e.g. LT50 or knockdown temperature; Wiesepape et al. 1972, Klok & Chown 2003). The other option is ramping of temperature and using endpoints. In the current experiments, ramping was used to heat or cool the animals at a rate of approximately 1 °C/minute. Other research which has used this method have debated on the different merits of a faster or slower ramping, but the current rate coordinates closely with other research on invertebrates (e.g. Buchanan et al. 1988, Calosi et al. 2008). Ramping was chosen not only due to the availability of equipment but

3.4. DISCUSSION

also because this type of experimentation can be viewed as more ecologically relevant as multiple endpoints can be determined as well as death. Whilst it is less likely that animals will suddenly experience such temperature drops (but it is possible, e.g. male *C. volutator* search the mud's surface for females), as compared to a more frequent daily increase and decrease of temperature with the rise and setting of the sun. As the results varied considerably, it is impractical to draw generalised conclusions, therefore each species will be discussed separately.

3.4.1 *C. volutator*

CTMax

On average, *C. volutator* was found to have a CTMax of 38.27°C in the summer, with a lower value of 37.91°C in the winter. The lowest CTMax was in winter over all salinities at 10°C, with the exception of the summer 10°C and 2 ppt combination. This seems intuitive, and may be an indication that animals reflect both the acclimation temperature and the seasonal variation. At optimal salinity (20 ppt), *C. volutator* showed a marked decrease in CTMax at 10°C between the summer and winter treatments. It appears that the animals lost their seasonal effect only in the winter when acclimated to 20°C. Work on the isopod *Sphaeroma rugicauda* (Leach) found similar results with a higher CTMax when acclimated to 20°C as compared to 5°C. A further decrease in thermal tolerance was found when animals acclimated to 5°C were exposed to extremely low salinities (< 1.7 ppt) (Marsden 1973). Acclimatory ability was not seen in the laboratory reared polychaetes, *Ophryotrocha diadema* (Akesson), however the interaction between temperature and salinity was noted to impact survival during prolonged exposure (Akesson & Costlow 1978). Salinity tolerance for these

3.4. DISCUSSION

polychaetes, was highest at lower temperatures (15 and 18°C), however optimal survival was determined to be at 25°C between 30 and 35 ppt (Akesson & Costlow 1978). It is possible that the current data for *C. volutator* is somewhat skewed as not all sexes and size combinations were represented by enough data. *Corophium volutator* requires further data on juvenile, males and females in order to fully understand where, if any, differences lie with respect to season and salinity for both juveniles and adults.

CTMin

In general, *C. volutator* has a lower CTMin in the winter when compared to summer, with an overall average of 3.84°C in the summer and 4.65°C in the winter. Specifically, the lowest CTMin is during the summer treatment at 20°C and 20 ppt, and highest again in the summer at 35ppt and 10°C. There are many possible explanations for this, however without further data this would be postulating. Further understanding may be gained from viewing the results in a more generalised fashion as trendlines of the temperature ranges.

Temperature range

During the summer the temperature range was greatest at 10°C and 35 ppt, yet during the winter treatment the same temperature and salinity combination has the smallest range with a negative slope. The information that can be derived from these plots is a general one as these are derived equations and the influence of mass is prevalent. Overall, large adults survived better in the 10°C and 35 ppt treatment over the summer than the winter. Yet in both seasons, the treatment closest to the animal's natural environment (20°C and 20 ppt) is very similar. Therefore, it appears that the temperature and salinity combinations are driving these generalised relationships.

3.4.2 *G. marinus*

CTMax

Gammarus marinus displayed a similar pattern to *C. volutator*, with winter and 10°C acclimation producing the lowest CTMax. A higher average CTMax of 36.61 °C in the summer was found compared to the winter average of 35.11 °C. *Gammarus marinus* has the lowest average CTMax of the three species, which might indicate its marine origin, or that its microhabitat is the least variable due to constant and heavy *Fucus* sp. cover, particularly during low tide.

CTMin

Regardless of acclimation temperature, the seasonal acclimation prevails as the smallest CTMin was found with the animals collected in the summer at 10°C, with the lowest CTMin at 10°C but in the winter treatment. On average, *G. marinus* had the lowest CTMin of all three species (−4.13°C summer and −7.22°C winter) which may be related to larger sized adults taking longer to cool as compared to extremely small *C. volutator* and *G. chevreuxi*. The lowest CTMin values were collected at 10°C and 35 ppt which might be reflective of this species' marine origins. Dorgelo (1977) working on the dessication response of *G. marinus*, found that, as in most amphipods there was a very poor response as there was no specific behavioural or physiological adaptations. A sudden drop in temperature at the beginning of spring is predicted to have a much smaller effect on the population compared to a sudden cold period at the end of summer owing to the species' acclimatisation to seasonal temperatures. Yet, these animals are often found exposed at low tide among the *Fucus* sp., which may be made possible due to the more chitinized exoskeleton preventing water loss (Dorgelo 1977).

3.4. DISCUSSION

This resistance to periodic dessication might be a factor in the increased resistance to cold found in these experiments by *G. marinus*, *Corophium volutator* and *G. chevreuxi* both live in microhabitats which have the possibility of remaining within small pools of water which is not possible where *G. marinus* is found. Therefore, the experiments being conducted in air may well be a contributing factor to the apparent higher tolerance to cold of *G. marinus*.

Temperature range

Gammarus marinus appears to have a smaller temperature range compared to *C. volutator*. All slopes were positive, indicating a strong correlation with mass. It would be predicted that if this was a simple relationships with a single factor (temperature or salinity) driving it, then one set combination of experimental conditions would prevail across the seasons. However, the largest temperature range was at 10°C and 35 ppt in the summer, but this did not reoccur in the winter, with 20°C and 20 ppt being largest. This points towards the complex relationship between salinity, acclimation temperature and season, where generalities are hard to draw.

3.4.3 *G. chevreuxi*

CTMax

Gammarus chevreuxi was found to have an average CTMax of 37.05°C in the summer and 36.42°C in the winter, but when the data are broken down a more complex pattern emerges. In general terms, the summer data at 10°C indicate a higher CTMax compared to winter 10°C, which in turn was higher than the winter 20°C data, with the summer 20°C having the lowest CTMax. This would suggest that the tempera-

3.4. DISCUSSION

ture acclimation was firstly influencing the high temperature tolerance then seasonal acclimation. *Gammarus chevreuxi* shows reduction in the effects of seasonality, whilst acclimation temperature appears to have a much stronger effect on determining CTMax. There is contrasting research concerning the effect, or lack thereof, of seasonality. For example, Lahdes & Vainio (2003) make reference to the loss of seasonal effects on CTMax when animals are kept in the laboratory for extended acclimation before experiments commence. Yet, Lagerspetz & Bowler (1993) found that there was no seasonal effect on CTMax for *Asellus aquaticus* (Linnaeus) which is in direct contrast to the crayfish *Orconectes rusticus* (Girard) which showed significant differences between seasonal acclimation (Layne et al. 1987).

CTMin

In general, these animals had a summer CTMin of -0.55°C and -0.65°C in the winter. These animals produced the highest CTMin in the winter treatment at 35 ppt and 10°C acclimation. This may be expected as this species is found in relatively freshwater streams, which rarely reach salinities near to that of seawater. As the CTMin was conducted in air, the possibility of false results being generated due to the salt particles increasing freeze tolerance can be eliminated. In the summer treatment, the animals showed the lowest CTMin at 20 ppt and 10°C and 2 ppt and 20°C . This might be a reflection of the natural conditions which these animals were found.

The only significant difference between males and females for the CTMin were found within the *G. chevreuxi* data. Males were found to have a significantly higher cold tolerance compared with females. Sexual dimorphism of *Gammarus* is well documented (Conlan 1991); likely driven by sexual selection as males physically attach themselves to females to mate-guard (Lincoln 1979, Conlan 1991). There is extensive research

3.4. DISCUSSION

on how body size can impact the heating and cooling rates of animals (for example lizards: Sepulveda et al. 2008, Rice et al. 2006). In general, larger animals cool more slowly than smaller ones. Yet, it is surprising that only one of the two *Gammarus* species showed any significant results.

Temperature range

Gammarus chevreuxi has a similar sized temperature range to *G. marinus*. The animals collected during the summer showed a relatively-straight forward relationship, in that the animals acclimated to higher temperatures had bigger temperature ranges than those acclimated to lower temperatures, regardless of salinity. This trend does not hold true for the animals collected in the winter. The trends follow an unexpected pattern, in that they group very closely together, with the highest salinities at both acclimation temperatures along with 20°C and 2 ppt with the largest difference between CTMin and CTMax. So the largest temperature ranges are present at extremes in salinity. From this data, it is apparent that *G. chevreuxi* does not live near its thermal extremes as the water in southern England rarely falls below zero nor reaches above 30°C. Therefore, if *G. chevreuxi* is able to live well within its optimal thermal zone, this might be a strategy for tolerating the changing salinities experienced in their natural environment.

In the case of the current experimentation, the results for *C. volutator* and *G. marinus* are significant, in that seasonal effects can be reversed in the winter but not in the summer. Although not investigated specifically here, it is possible that the two-day acclimation of winter animals at 20°C was long enough to induce the production of heat shock proteins. Equally, it is possible that the summer animals which were already producing these proteins were not acclimated at 10°C for long enough to stop the production as previous research has shown an estimated half-life of approximately

3.4. DISCUSSION

2 days (Landry et al. 1982). Sorte & Hofmann (2005) acclimated their *Nucella* species for three weeks prior to experimentation so that cells could fully turnover the heat-shock molecules. It should be pointed out that thermal acclimation and the production of stress proteins is distinct from heat shock. Thermal adaptation was also seen in a terrestrial isopod (*Porcellio laevis* (Latreille)) from North and central Chile, where the population from desert regions showed a tolerance to high temperatures in contrast to the colder southern population which had a much greater tolerance to low temperature (Castaneda et al. 2004). Korhonen & Lagerspetz (1996) studied heat shock, acclimation and thermal maximum in the isopod, *Asellus aquaticus*. Their results showed that the thermal maximum of the isopod was significantly higher if the animal was previously rapidly heat shocked, but this acclimation effect only lasted for 6 hours. However, animals which were acclimated to higher temperatures for longer periods showed an extended elevation of thermal maximum compared with animals which were rapidly shocked (Korhonen & Lagerspetz 1996). From this work, Korhonen & Lagerspetz (1996) found that at least in part, heat-shock and thermal acclimation are derived from two separate mechanisms in *A. aquaticus*. Similarly, the subterranean amphipod *Niphargus rhenorhodanensis* (Schellenberg), was able to survive innoculative freezing when it was acclimated in cold temperatures, whereas the gammarid *Gammarus fossarum* (Koch) was not (Issartel et al. 2006), indicating interspecific variability in acclimatory ability. This was hypothesised to be an adaptation retained from early glaciations where it was possible that some ice-cold water penetrated the sediment (Issartel et al. 2006). In the current experiments, thermal acclimation was the focus by keeping animals for two days prior to any manipulation.

Behavioural adaptations can play a large role in determining, on an individual scale,

3.4. DISCUSSION

what temperature regime animals are exposed to (Sepulveda et al. 2008, Andrews 1998, Gaston & Spicer 1998). Male *C. volutator* explore burrows in order to find mates and therefore could potentially be exposed to much colder winter temperatures and much warmer summer temperature. There is a considerable amount of research on behavioural adaptations of different life stages. For example, lizards have been shown to thermoregulate using mainly behavioural adaptations (Sepulveda et al. 2008). Sepulveda et al. (2008) noted that behavioural adaptations were a strategy adapted by all age classes studied. Juvenile lizards were the first to emerge from the burrows in the morning and the adults were last to leave in the evening. This is why broad or 'macro' generalisations should be tempered with consideration of the individual animal and its life stage as this could be a large determinant of the chosen habitat.

3.4.4 Conclusions

In terms of future work, Hoffmann & Blows (1994) make a feasible argument for the use of transplantation experiments. It is impossible to completely replicate the natural environment in the laboratory, particularly the microhabitat temperature ranges, on a tidal and monthly basis. Therefore, the transplantation experiment similar to MacNeil et al. (2004) but considering (both local and geographic distributions) combined with known physiological limits, can perhaps give us a more complete and accurate picture for the causes and limitations of a species distribution. From the current research as well as previous work, critical thermal maximum and minimum are important not only for the understanding of the biology of individual species, but also can be used to elucidate the factors effecting the distribution from an ecological perspective in a changing climate. More pragmatic applications should also be considered such as the potential for reduction in stress resulting in increased production during aquaculture

3.4. *DISCUSSION*

(e.g. Rodriguez et al. 1996, Wiesepepe et al. 1972).

Chapter 4

Study of the abundance and distribution of the three amphipod species

4.1 Introduction

The search to explain patterns in biology is an ancient pursuit; many theories attempting to explain these patterns have become popular and are generally based in apparently simple and logical mechanisms (Gaston & Chown 1999). According to del Rio (2008), there are two perspectives within science. The received view where there are generalised theories or ‘laws’ to explain patterns, and the model-centric view, a mathematical approach to each investigation. A persistent pattern that has been present throughout the development of ecology is the positive relationship between abundance and distribution of organisms (Darwin 1859, Gaston et al. 2000). The approach taken has been the received view, with the belief that analysis of current and previous research will result in the discovery of the mechanisms driving this relationship for all species. Many explanations for the positive relationship between abundance and occupancy have been proposed (see Chapter 1, Gaston et al. 1997).

Much of the research on abundance and distribution has focused on the terrestrial environment due to the availability of large datasets for birds and plants (e.g. Gaston et al. 1997, Gaston, Blackburn, Gregory & Greenwood 1998, Ozinga et al. 2004). How-

4.1. INTRODUCTION

ever, this research is limited in its applicability with regards to the marine environment due to different dispersal methods and effect size (Blackburn et al. 2006). Some research, however, has looked at this macroecological pattern with marine species (e.g. Foggo et al. 2007, Webb et al. 2009). As marine environments can be considered to be homogenous environments (Blackburn et al. 2006), this research cannot necessarily be compared to the unique set of conditions which are found in estuaries. Estuarine habitats are dichotomous environments, they can be both very dynamic at the zone of interaction with freshwater and marine, but the species diversity can also be the same as the marine or freshwater habitat they are derived from, as the animals are limited by their physiological tolerances (Bilton et al. 2002). Despite these different environmental conditions, Foggo et al. (2003) and Webb et al. (2009) found abundance-occupancy relationships in U.K. estuaries to be similar to terrestrial and freshwater organisms (Blackburn et al. 2006). However, further research, on key species within the macroinvertebrate assemblage is necessary to fully explore the relationship between abundance and distribution in estuaries.

Amphipods are known to influence the structure of macroinvertebrate communities as prolific 'scrapers' and 'shredders' (MacNeil et al. 1997). Many factors are expected to limit amphipod distribution including physico-chemical parameters (MacNeil et al. 2000), resource availability (Bettison & Davenport 1976, MacNeil et al. 2000), substrate, and reproduction (Bettison & Davenport 1976). *Corophium volutator* is an important and abundant amphipod species as prey for commercially important fish (Hampel et al. 2009), and has been shown to destabilise mud flats due to extensive bioturbation of the sediment, excluding species such as pioneer vegetation (Hughes & Gerdol 1997), although there is some doubt as to the strength of this effect (Limia &

4.1. INTRODUCTION

Raffaelli 1997). The patchy distribution of *C. volutator* has been the subject of much research (e.g. Campbell & Meadows 1974, Flach 1992, Lawrie et al. 2000). Various explanations have been proposed to explain this patchiness. *Corophium volutator* was shown to prefer anaerobic mud to aerobic, but was not limited by food availability (Meadows 1964a). However, Bell et al. (1997) found food availability to limit *C. volutator*'s distribution. Further to this both tidal and residual currents as well as hydrodynamic factors can affect the distribution of sediment, including distribution of juvenile animals (Lawrie et al. 2000). Other publications have added to an already complex picture, showing predation (Peer et al. 1986, Hughes & Gerdol 1997, Flach & Debruin 1993, Jensen & Andre 1993), environmental variability (McLusky 1967, 1968, 1970, McLusky et al. 1986, Meadows & Ruagh 1981) and level of exposure (Hughes & Gerdol 1997) to have significant impacts on *C. volutator*'s distribution.

Gammarid amphipods are a common sub-order, found throughout the U.K. (Lincoln 1979). This group of amphipods are thought to feed primarily on decaying organic matter, but research has shown that some species may be active predators and this could be a factor determining their distribution (MacNeil et al. 1997, Dick et al. 2005). More specifically, *Gammarus marinus* has been the focus of a variety of research and various mechanisms have been proposed to explain its current distribution including: presence of prey species (Dick et al. 2005), presence of suitable habitat (e.g. patches of Furoid seaweeds), extremes in salinity, avoidance of industrial runoff and reduced influence of tidal flow (Maranhao et al. 2001).

Previous work on the abundance and distribution of *G. chevreuxi* is limited (Subida et al. 2005). What is known is that population abundance can vary widely and is affected by environmental factors, particularly salinity (Barnes et al. 1979, Girisch

4.2. MATERIAL AND METHODS

et al. 1974, Dieleman 1978), predation (Barnes et al. 1979), competition with other *Gammarus* species (Barnes et al. 1979), dissolved oxygen and food availability (Subida et al. 2005).

The aim of this chapter was to quantify the spatial and temporal variability in the abundance and distribution of the three amphipod species. This was achieved by monitoring the abundance and distribution of these amphipods at sites along three estuaries over the course of 24 months. In the current chapter, multiple questions were posed: does the abundance and occupancy of these amphipods change over time? Do all sites sampled show the same population density? Is the composition (juvenile:adult and male:female) of these populations similar across all sites and sampling dates? From these data, measures for population persistence and abundance, both within- and between- patches (sites and estuaries) were determined. These data will later be related to physiological parameters derived from the previous chapters in the final model synthesis section of the thesis. The central question remains: can these physiological limits be used to predict the presence or absence of amphipods given population data and habitat information?

4.2 Material and Methods

4.2.1 Site and sampling design

For each species, three sampling sites per estuary were chosen, based on their accessibility, the presence of habitat corresponding to each species of amphipod, and preliminary sampling results. The Taw (SS 530 310)/Torridge (SS 490 260) estuary in North Devon and the Plym estuary (SX 540 566) in South Devon were chosen as study

4.2. MATERIAL AND METHODS

sites. When a preliminary study found that *C. volutator* was absent from in the Plym estuary even when it has been reported there previously (J.I. Spicer, pers. comm.), it was necessary to add the Avon estuary (SX 666 439). For *C. volutator*, two estuaries were used; the Avon and the Taw/Torridge. For the *Gammarus* sp., the Avon and the Plym estuaries were chosen. Sampling occurred every two months from June 2006 to June 2008.

Corophium volutator were collected by scraping the top 1 cm layer of sediment from 5 randomly selected 1m² quadrats in each site at each date. This material was then roughly sieved through a 500 μ m mesh bags *in situ* and the animals were brought back to the laboratory in bags with sediment and water. When back in the laboratory, the samples were sieved again through a 250 μ m (to ensure no individuals were lost with the removal of sediment) and fixed in a 4% buffered (in seawater) formalin solution. This method was chosen after extensive trials with coring. The 10 cm PVC cores (to a depth of 10 cm) traditionally used in studies of soft sediment fauna (e.g. Frid & James 1989) were found not to reflect the actual densities of *C. volutator* seen in the field as the area of material collected per unit volume of sediment processed was low. The scraping method has been used previously to collect *Corophium curvispinum* (Harris & Musko 1999). However, in order to properly assess the effectiveness of these scrape samples, one core sample was collected at every site at every date for comparison with the scrapes to ensure that a portion of the population at depths not reached by scraping was not missed, and to validate population estimates against this more commonly employed sampling system.

An additional dataset was made available for *C. volutator* from the Avon estuary. These data were collected using 10x10 cm cores, with 80 samples collected across the entire

4.2. MATERIAL AND METHODS

estuary during July 2007. The samples were returned to the laboratory where they were sieved and animals were individually removed and preserved in 70% ethanol for identification. One sample was lost and thus 79 data points were generated. These data were used to further validate the statistical methods used (see Results - Cores versus scraping methodology).

Gammarus marinus were collected using 'fake seaweed' traps which consist of slate tiles (50 x 50 cm) covered with 100g of shredded black refuse bags, secured with a 1x1 cm mesh covering (see fig. 4.1), with 5 tiles at each site. This method was chosen after several other collection methods were shown to be ineffective, including both hand capture and tile traps. The shredded bags were removed during sampling sessions and all animals taken back to the laboratory, in order for the refuse bags to be rinsed through a 250 μ m sieve to retain all individuals. Animals were then fixed in a 4% formalin solution.



Figure 4.1: Example of slate tile covered in mesh which secured the shredded refuse bags used to collect *G. marinus*

4.2. MATERIAL AND METHODS

Gammarus chevreuxi were collected in streams using kick sampling methods; a standard FBA net (500 μm mesh size) was placed on the river bed and a delineated area (25 x 20 cm) was kicked for one minute; 5 samples per site were collected at each estuary. The samples were sieved *in situ* in a 500 μm mesh bag and all animals brought back to the laboratory with sediment. All samples were rinsed through a 250 μm sieve and sediment removed before being fixed in a 4% buffered formalin solution.

Each preserved sample was emptied into a sorting tray and all visible amphipods were removed by hand and preserved in 80% ethanol. Once all 1105 samples were processed, the samples were examined for species identification. The samples which remained were then organised according to species and date. Each sample was then enumerated and any samples which exceeded 200 individuals were subsampled using a Folsom plankton splitter (see fig. 4.2), allowing extrapolation of densities from tractable subsamples.

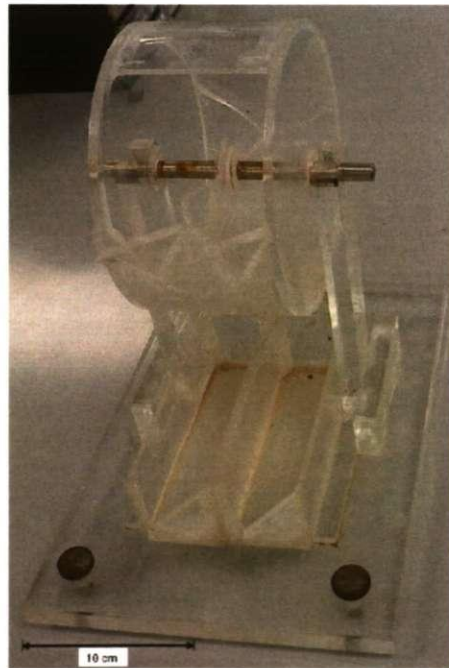


Figure 4.2: Plankton splitter used to divide large samples of amphipods

4.2. MATERIAL AND METHODS

Each individual amphipod was sexed and length measured. The density was determined per sample and per m².

Previous research has assigned animals to different categories according to life stage and size:

1. For *C. volutator* the categories were based on size: juveniles being smaller than 2mm, with males and females being split into different size classes (2-4 mm, 4-6 mm and 6-8 mm), with the largest animals being above 8 mm (Lawrie et al. 2000).
2. Categories for *G. marinus* were less clear, but have previously been considered as recently hatched, males and females (Ford et al. 2003).
3. For *G. chevreuxi* previous research split the animals into the following categories: juveniles, immature females, incubating females, empty females, immature females and mature males (Subida et al. 2005).

Taking the principles of this research, standard categories were developed and used for all three species:

1. Juveniles - <2 mm for *C. volutator* and *G. chevreuxi* and <3 mm for *G. marinus*.
2. Male - Presence of penial papillae for *C. volutator* (McCurdy et al. 2004) and size and shape of gnathopod for *Gammarus* (see fig. 4.3).
3. Female - no visible oostegites.
4. Female - small oostegites without setae (e.g. Watkin 1941).

4.2. MATERIAL AND METHODS

- 5. Female - large oostegites with setae (e.g. Watkin 1941).
- 6. Female - with eggs.
- 7. Female - with developing juveniles (segmented embryos, appendages segmented and eye spot, see: Subida et al. 2005, Marques & Nogueira 1991).

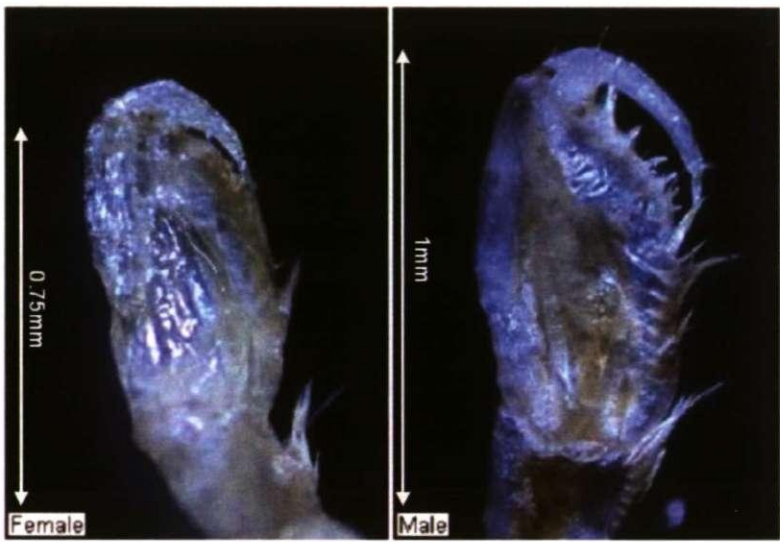


Figure 4.3: Gnathopods of adult male and female *Gammarus* for determination of sexes

4.2.2 Statistical analysis

Some sites were found to consistently lack the target species of *Gammarus*, despite ideal habitat location and presence of animals in preliminary sampling. Instead of including these ‘empty’ sites, which disproportionately skewed analysis, it was decided that a reduction in the numbers of sites was preferable to including null data. This meant that the data presented here for *G. marinus* were taken from two sites in each of the estuaries and the data for *G. chevreuxi* were taken from two sites on the Avon and three on the Plym estuary. Therefore, a total of 11 sites across the three estuaries

4.2. MATERIAL AND METHODS

(see table 4.1) were analysed every other month from June 2006 to June 2008. The data used for density (ind./m²) figures were log₁₀ transformed to reduce the variation encountered.

Table 4.1: Location of sampling sites with Ordinance Survey coordinates indicating where samples were collected for each amphipod species

Estuary	Site	<i>C. volutator</i>	<i>G. marinus</i>	<i>G. chevreuxi</i>
Avon	Upper stream (SX 692 472)	x		x
	Tidal Road (SX 683 468)	x	x	x
	Main channel (SX 676 464)	x		
	Hexdown (SX 673 448)		x	
Taw	Upper stream (SS 495 325)	x		
	Salt marsh (SS 517 334)	x		
	Main channel (SS 544 326)	x		
Torridge	Main channel (SS 457 261)	x		
Plym	Chelson Meadow (SX 503 549)		x	x
	Dam (SX 516 556)		x	x
	Upper stream (SX 524 565)			x

To determine if there were differences between densities, sex ratios and juvenile to adult ratios (J/A ratio) across sites and sampling dates, analyses were conducted in SPSS ver. 16.0 (SPSS Inc. Chicago). Densities were first log₁₀ transformed to normalise analytical residuals. Due to the imbalanced sampling design, estuaries were not used as a factor in the analysis. Instead, site was used as a fixed factor in the analysis,

4.3. RESULTS

with date being used as a random factor. Univariate ANOVA was used to determine effects of site and date on the populations. The strength of significant effects were assessed using F-values combined with the effect size (partial η^2); variance components were calculated for all terms involving random effects to allow comparisons amongst species. This analysis was conducted on each species individually. Thus, spatial (between sites), temporal (between dates) and spatio-temporal (site by date interaction) variability could be comparatively quantified.

To investigate the patchiness of the Avon population of *C. volutator*, the degree of spatial autocorrelation in the density data was determined for the additional Avon dataset from July 2007. An index of dispersion was conducted to measure the dispersion of the density which indicates what kind of pattern was present (aggregated, random or uniform). Further to this, from a selection of metrics available for undertaking an estimation of spatial patterning (Legendre & Legendre 1998), correlation between a triangular distance matrix (pair-wise Euclidean geographic distances between samples) and a second similarity matrix (Euclidean distances between the sample counts of species in the same samples) was chosen (A. Foggo, pers. comm. August 2009). This matrix correlation was conducted in Primer ver. 6.0 (Primer-e, Ivybridge, U.K.) using Spearman's rank correlation coefficient ρ . The analysis was run initially including all samples regardless if they contained animals ($n = 79$), and was run a second time excluding the samples which did not contain animals ($n = 34$).

4.3 Results

Cores versus scraping methodology Some previous researchers have used coring to a depth of between 2 and 10 cm as the typical method for collecting and quantifying

4.3. RESULTS

Corophium populations (Flach & Debruin 1993, McLusky 1968, Hilton et al. 2002, Lawrie et al. 2000, Möller & Rosenberg 1982, Crewe et al. 2001, Flach 1992). As stated earlier, to ensure the scraping method employed in the current work was properly representing the *Corophium* densities, a 10 cm core was also collected. The results of these cores can be considered broadly incomparable to the scraping method. Densities were similar; cores were found to have densities between 0 and 1655 ind./m², whereas scrapes were found to range between 0 and 1162 ind./m². However, *Corophium* were only found in 6.6% of the coring samples whereas 36.9% of scrapes contained individuals. Furthermore, the size frequency distribution in the cores was highly right skewed, unlike that of the scrape samples. It appears that the greater surface area provided by the scrapes reduces the variability in the samples, which would not be possible without a great increase in numbers of cores and amount of sediment processed.

The additional dataset from July 2007 was tested for spatial patterns. The index of dispersion was high, and this resulted in a high χ^2 value (1660.949), which indicated a highly aggregated pattern. The results of the PRIMER analysis showed a significant degree of autocorrelation when the null data were incorporated ($\rho = 0.181$, $n = 79$, $P = 0.003$), possibly due to type 1 error derived from correlations between shared zeros. When the analysis was run without the sites lacking animals, the results suggested a lack of any spatial patterning ($\rho = -0.081$, $n = 34$, $P > 0.05$). Overall, the scraping method was considered to be more than adequate and the data used in the main analysis were only those collected with this method.

C. volutator Density significantly differed between sites (see table 4.2 and fig. 4.4). Date was also found to be significant ($P = 0.002$) and explained 15.32% of the variation attributable to the random factor; this increased to 48.94% for the interaction between

4.3. RESULTS

date and site (see table 4.2). Site and date alone were not found to be significant for the sex ratio or the J/A ratio (see tables 4.3 and 4.4). However, when site and date interacted, a significant ($P<0.001$) result was found for both sets of ratios, with 48.45% of the variation explained for sex ratio and 25.5% for J/A ratio.

Five individual months were found to have very high densities compared with the rest of the sampling period, but no pattern was apparent (see fig. 4.5). Females dominated all samples (see fig. 4.6) and there did not appear to be any seasonal pattern in the abundance of juveniles (see fig. 4.7). However, there did appear to be an increase in juveniles when the density of males decreased in October 2007 (see fig. 4.7). The Taw estuary site, 'main channel' had extremely high densities compared to all other sites (see fig. 4.5).

Table 4.2: Results of the ANOVA of log density of *C. volutator*.

	DF	MS	F	Significance	Partial η^2	Variance
Intercept	1	591.528	52.879	<0.001	0.815	
Error	12	11.187				
Site	6	23.165	6.021	<0.001	0.334	
Error	72	3.847				
Date	12	11.187	2.908	0.002	0.326	15.32%
Error	72	3.847				
Site x Date	72	3.847	7.847	<0.001	0.608	48.94%
Error	364	0.490				35.74%

Table 4.3: Results of the ANOVA of the sex ratio of *C. volutator*.

	DF	MS	F	Significance	Partial η^2	Variance
Intercept	1	38.898	126.501	<0.001	0.894	
Error	15.063	0.307				
Site	6	6.391	1.312	0.268	0.127	
Error	54.134	0.298				
Date	11	0.370	1.187	0.319	0.200	3.09%
Error	52.221	0.311				
Site x Date	47	0.360	4.004	<0.001	0.528	48.45%
Error	168	0.090				48.45%

4.3. RESULTS

Table 4.4: Results of the ANOVA of the juvenile to adult ratio of *C. volutator*.

	DF	MS	F	Significance	Partial η^2	Variance
Intercept	1	57128.926	2.340	0.148	0.139	
Error	14.526	24415.416				
Site	6	17060.088	1.017	0.423	0.093	
Error	59.502	16774.772				
Date	11	29308.136	1.705	0.096	0.250	17.45%
Error	56.419	17187.137				
Site x Date	47	18842.576	2.089	<0.001	0.358	25.5%
Error	176	9018.699				57.05%

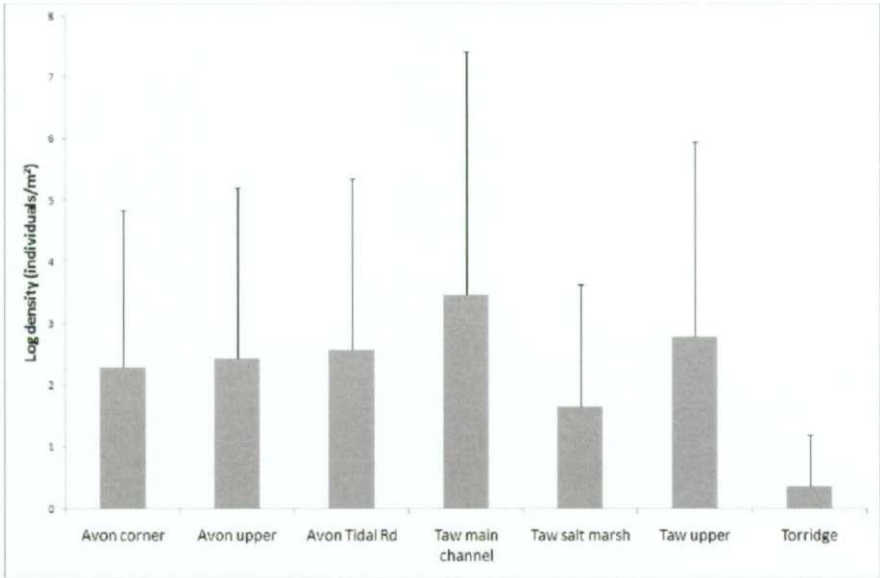


Figure 4.4: Mean (\pm s.d.) log density (ind./m²) of *C. volutator* at different sites, n = 65 for each site.

4.3. RESULTS

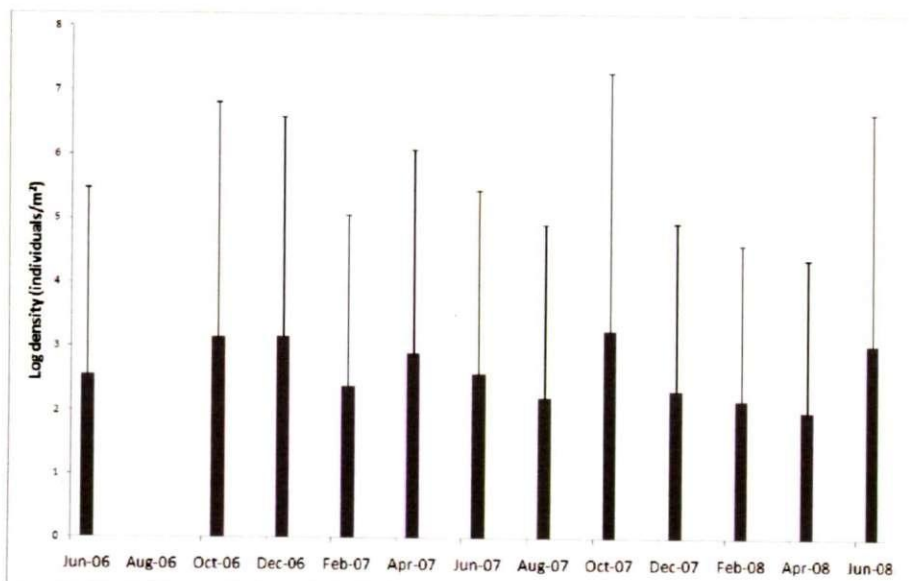


Figure 4.5: Mean (\pm s.d.) log density (ind./m²) of *C. volutator* at different dates, n = 55 for each date.

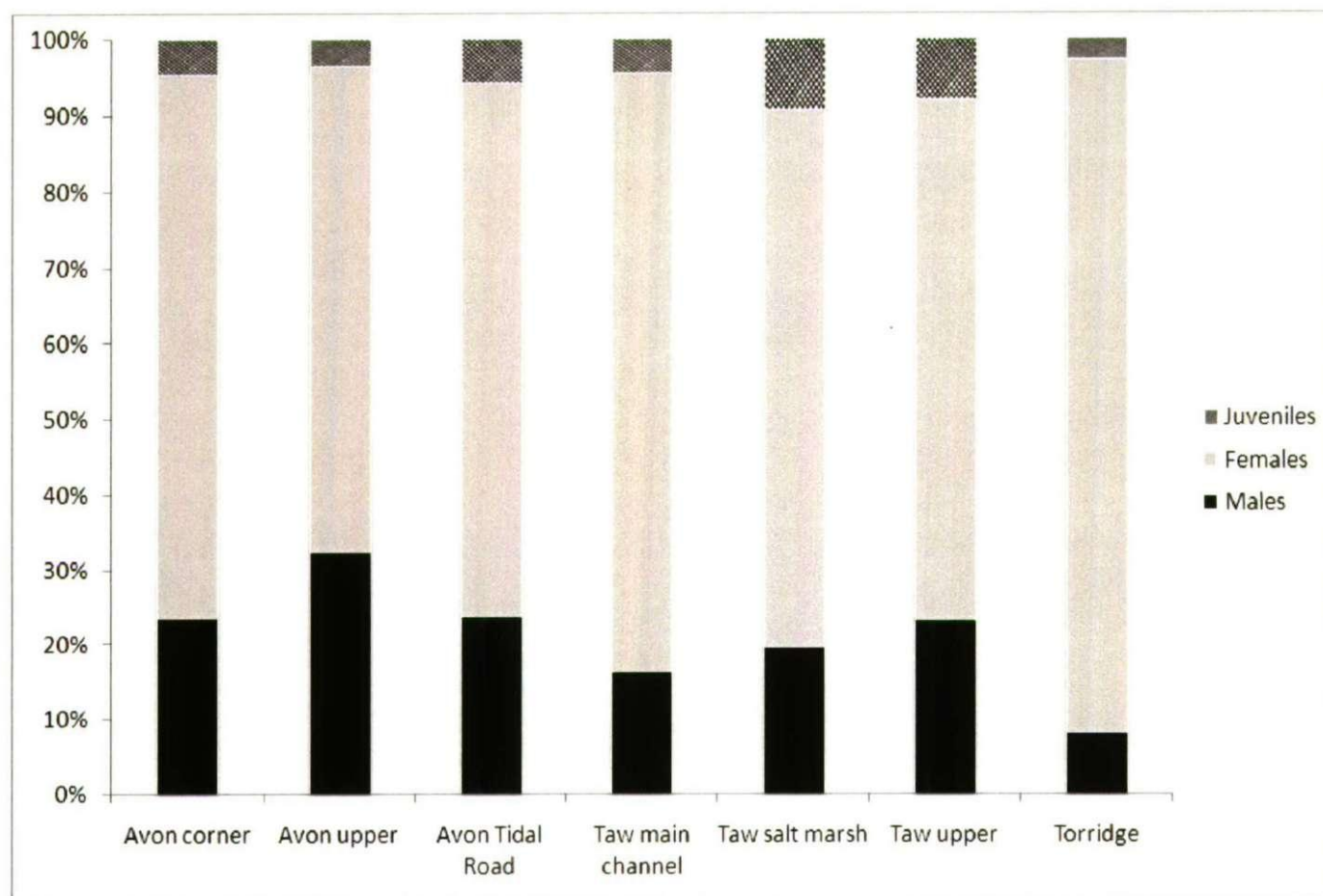


Figure 4.6: Mean proportional percentage abundance (ind./m²) of juvenile, female and male *C. volutator* at different sites.

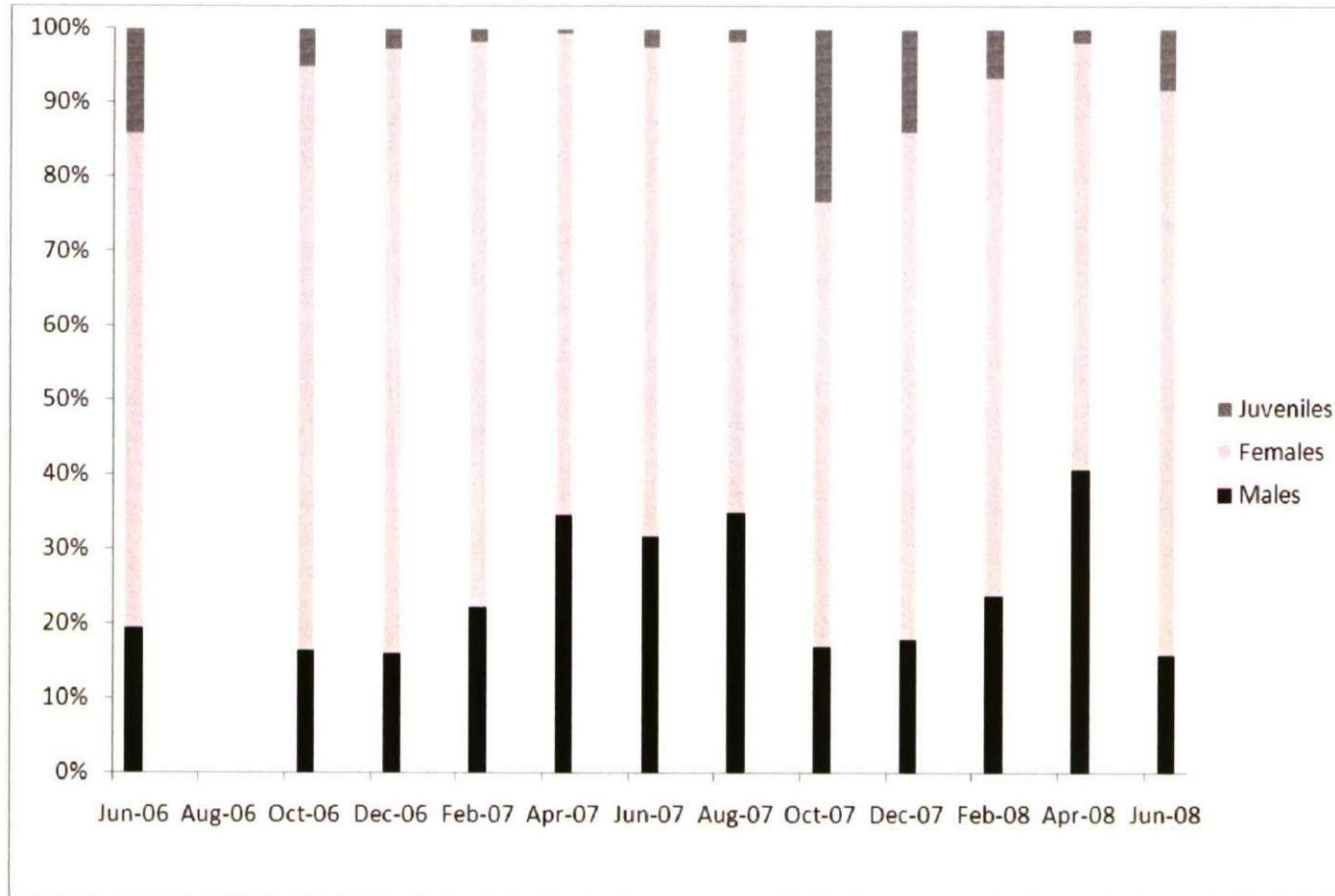


Figure 4.7: Mean proportional percentage abundance (ind./m²) of juvenile, female and male *C. volutator* at different dates.

4.3. RESULTS

G. marinus Again, density significantly differed between sites (see table 4.5). Date was also found to be significant ($P = 0.002$) and explained 30.13% of the attributable variation; the interaction between date and site ($P < 0.001$) explained 16.67%. Site and date were not found to be significant for the sex ratio or the J/A ratio, and no significant result was found when the factors interacted (see tables 4.6 and 4.7).

The Plym estuary ‘Chelson Meadow’ site had the highest density of *G. marinus* of all sites (see fig. 4.8). The second year of sampling shows much higher densities (with the exception of October 2007) than the first (see fig. 4.9). Females dominated all samples with very few juveniles present at the Plym ‘Dam’ site (see fig. 4.10). Reproduction does not appear to be linked with date or site, however, abundance of males was seen to peak in February of both years (see figs. 4.10 and 4.11).

Table 4.5: Results of the ANOVA of log density of *G. marinus*.

	DF	MS	F	Significance	Partial η^2	Variance
Intercept	1	157.833	17.972	0.001	0.600	
Error	12	8.782				
Site	3	6.642	4.095	0.013	0.254	
Error	36	1.622				
Date	12	8.782	5.414	<0.001	0.643	30.13%
Error	36	1.622				
Site x Date	36	1.622	2.568	<0.001	0.308	16.67%
Error	208	0.632				53.20%

Table 4.6: Results of the ANOVA of the sex ratio of *G. marinus*.

	DF	MS	F	Significance	Partial η^2	Variance
Intercept	1	18.013	128.679	0.001	6.882	
Error	17.275	0.140				
Site	3	0.072	6.525	0.669	0.052	
Error	28.702	0.138				
Date	10	0.145	1.056	0.426	6.271	0%
Error	28.337	0.138				
Site x Date	21	0.141	1.174	0.305	0.284	4%
Error	62	0.120				96%

4.3. RESULTS

Table 4.7: Results of the ANOVA of the juvenile to adult ratio of *G. marinus*.

	DF	MS	F	Significance	Partial η^2	Variance
Intercept	1	0.044	2.882	0.095	0.046	
Error	59.953	0.015				
Site	3	0.008	0.465	0.708	0.022	
Error	62.625	0.016				
Date	10	0.007	0.416	0.934	0.064	0%
Error	61.169	0.016				
Site x Date	21	0.010	0.206	1.000	0.065	24.19%
Error	62	0.048				75.84%

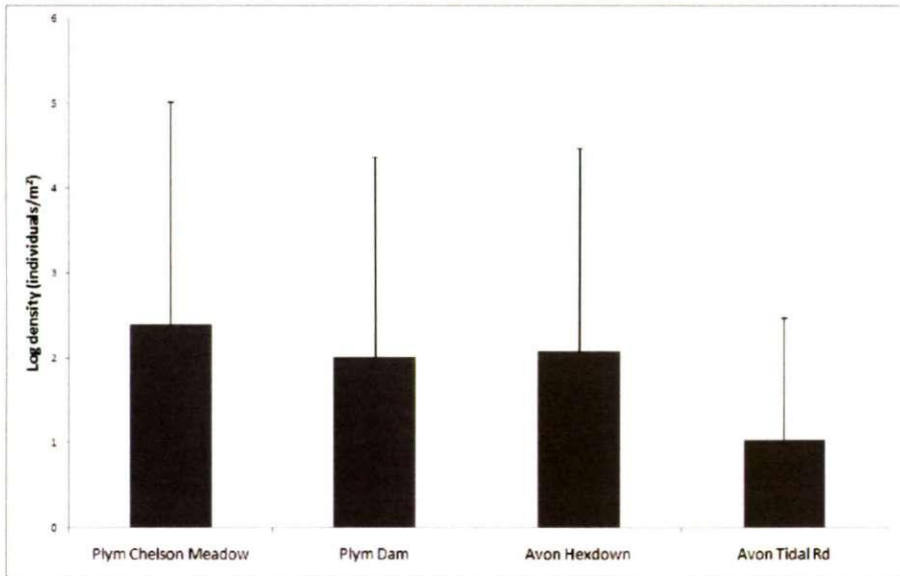


Figure 4.8: Mean (\pm s.d.) log density (ind./m²) of *G. marinus* at different sites, n = 65 for each site.

4.3. RESULTS

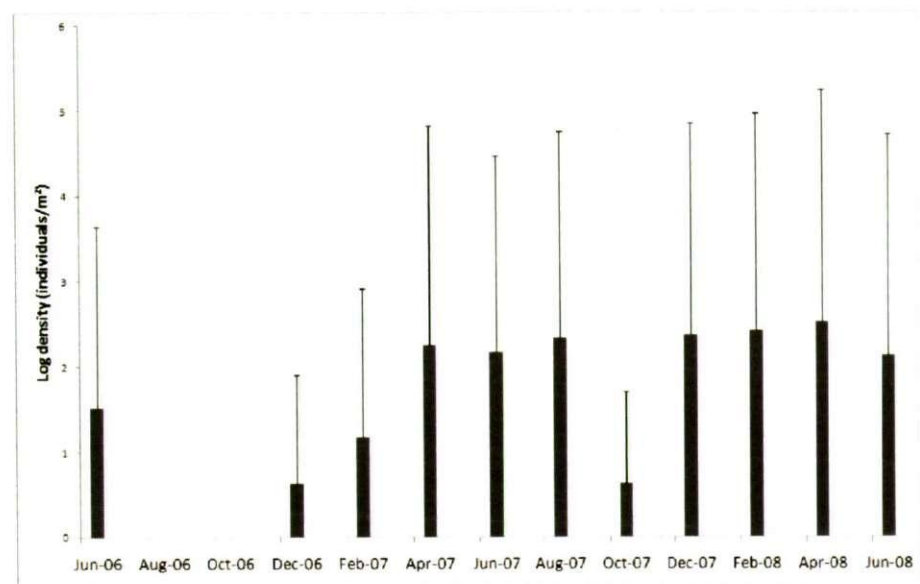


Figure 4.9: Mean (\pm s.d.) log density (ind./m²) of *G. marinus* at different dates, n = 55 for each date.

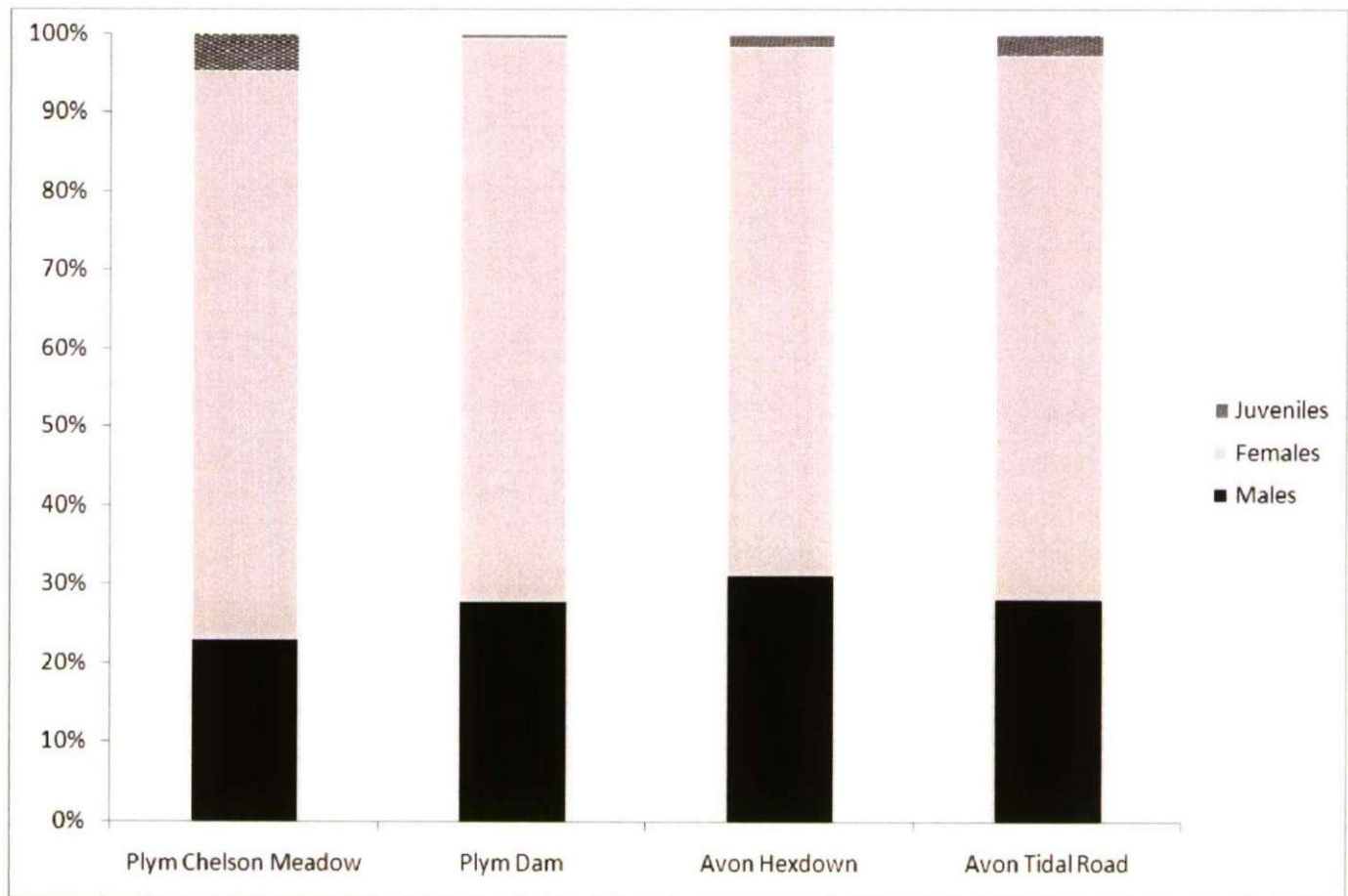


Figure 4.10: Mean proportional percentage abundance (ind./m²) of juveniles, females and males *G. marinus* at different sites.

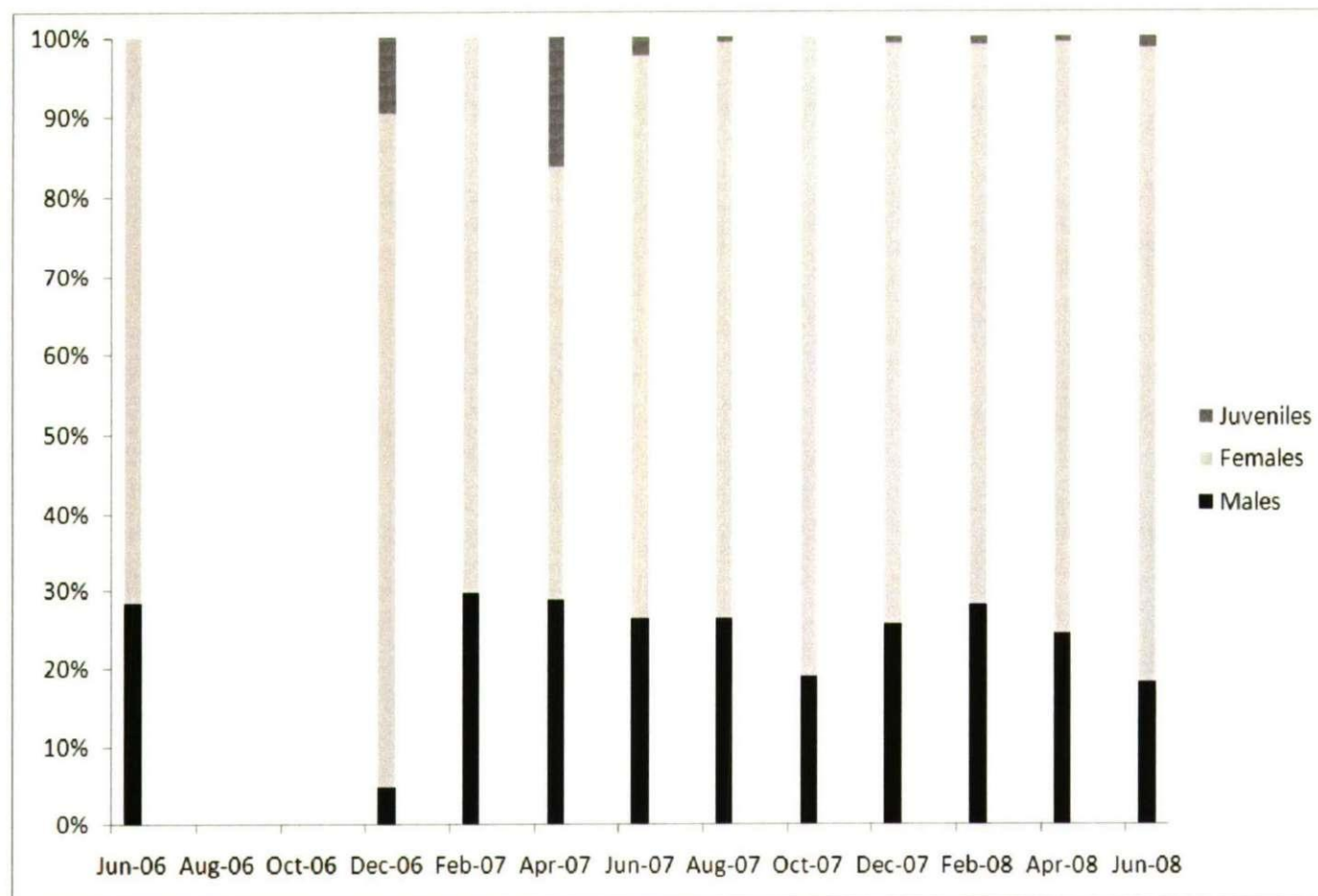


Figure 4.11: Mean proportional percentage abundance (ind./m²) of juvenile, female and male *G. marinus* at different dates.

4.3. RESULTS

G. chevreuxi As with the other two amphipod species, the interaction between site and date was significant (see table 4.8). Date was also found to be significant ($P = 0.001$) and explained 25.92% of the variation and when site interacted with date, 43.32% of the attributable variation could be explained by the random factor ($P < 0.001$). Site and date were not found to be significant for the sex ratio (see table 4.9) or the J/A ratio (see table 4.10). However, the interaction between site and date was significant ($P < 0.001$) in both sets of ratios, with 36.13% of the attributable variation accounted for in sex ratio and 59.45% for J/A ratio.

The Avon estuary ‘upper stream’ had the highest density across all sites (see fig. 4.12). The density of *G. chevreuxi* appears to increase towards the summer months with October and December showing very low densities (see fig. 4.13). Females dominated all samples, with only the Avon ‘Tidal Road’ site with a ratio close to 1:1 for males and females (see fig. 4.14). Release of juveniles from the brood pouch appeared to be during the summer months, as an increase in juvenile abundance was apparent every June sampling date (see fig. 4.15).

Table 4.8: Results of the ANOVA of log density of *G. chevreuxi*.

	DF	MS	F	Significance	Partial η^2	Variance
Intercept	1	249.316	25.523	<0.001	0.680	
Error	12	9.768				
Site	4	13.986	5.183	0.001	0.302	
Error	48	2.699				
Date	12	9.768	3.620	0.001	0.475	25.92%
Error	48	2.699				
Site x Date	48	2.699	8.033	<0.001	0.597	43.32%
Error	260	0.336				30.77%

4.3. RESULTS

Table 4.9: Results of the ANOVA of the sex ratio of *G. chevreuxi*.

	DF	MS	F	Significance	Partial η^2	Variance
Intercept	1	17.465	58.967	<0.001	0.814	
Error	13.5	0.296				
Site	4	0.245	0.859	0.500	0.109	
Error	28.128	0.285				
Date	10	0.331	1.127	0.379	0.294	3.14%
Error	27.037	0.294				
Site x Date	24	0.326	2.860	<0.001	0.39	36.13%
Error	104	0.114				60.73%

Table 4.10: Results of the ANOVA of the juvenile to adult ratio of *G. chevreuxi*.

	DF	MS	F	Significance	Partial η^2	Variance
Intercept	1	179.487	2.741	0.123	0.180	
Error	12.466	65.476				
Site	4	86.978	1.374	0.270	0.171	
Error	26.595	63.307				
Date	10	72.516	1.141	0.371	0.301	12.16%
Error	26.554	63.553				
Site x Date	25	74.933	7.008	0.001	0.625	59.45%
Error	105	10.692				28.39%

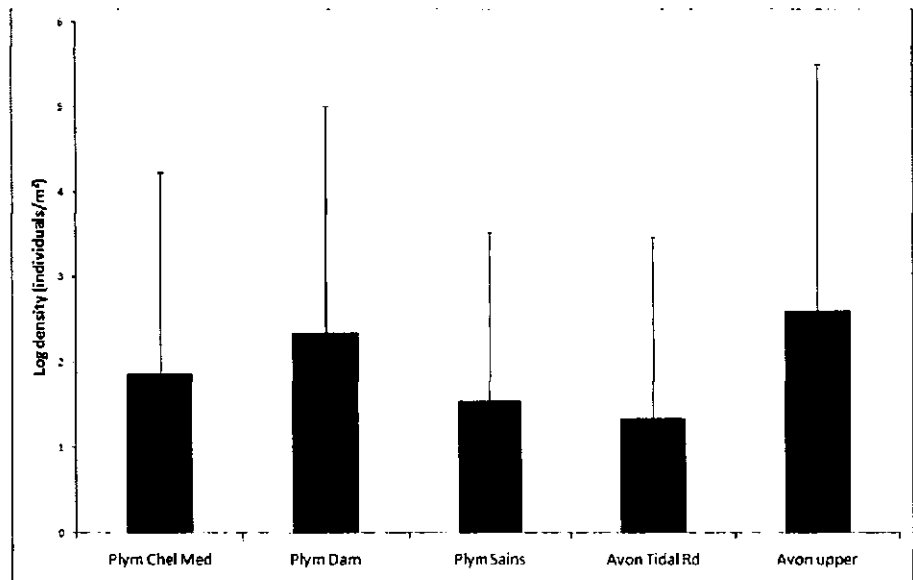


Figure 4.12: Mean (\pm s.d.) log density (ind./m²) of *G. chevreuxi* at different sites, n = 65 for each site.

4.3. RESULTS

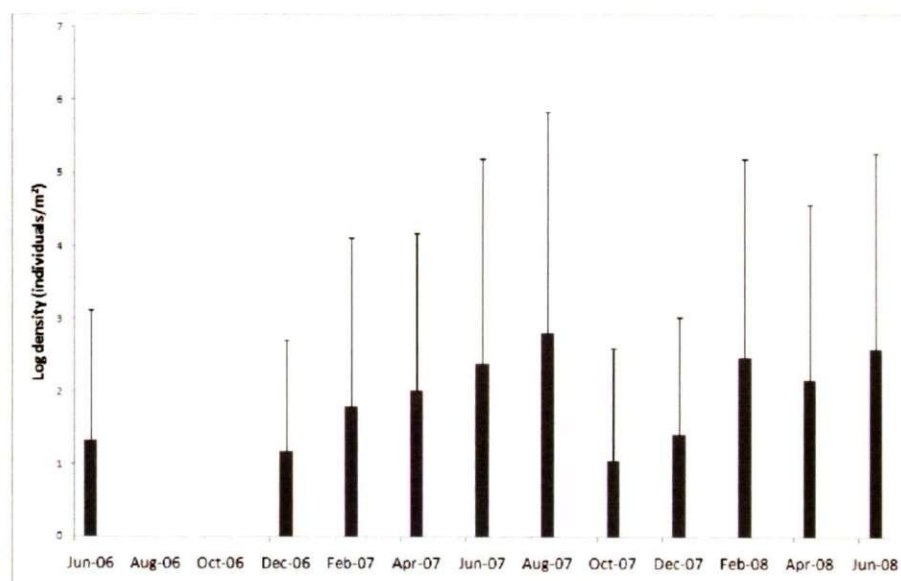


Figure 4.13: Mean (\pm s.d.) log density (ind./m²) of *G. chevreuxi* at different dates, n = 55 for each date.

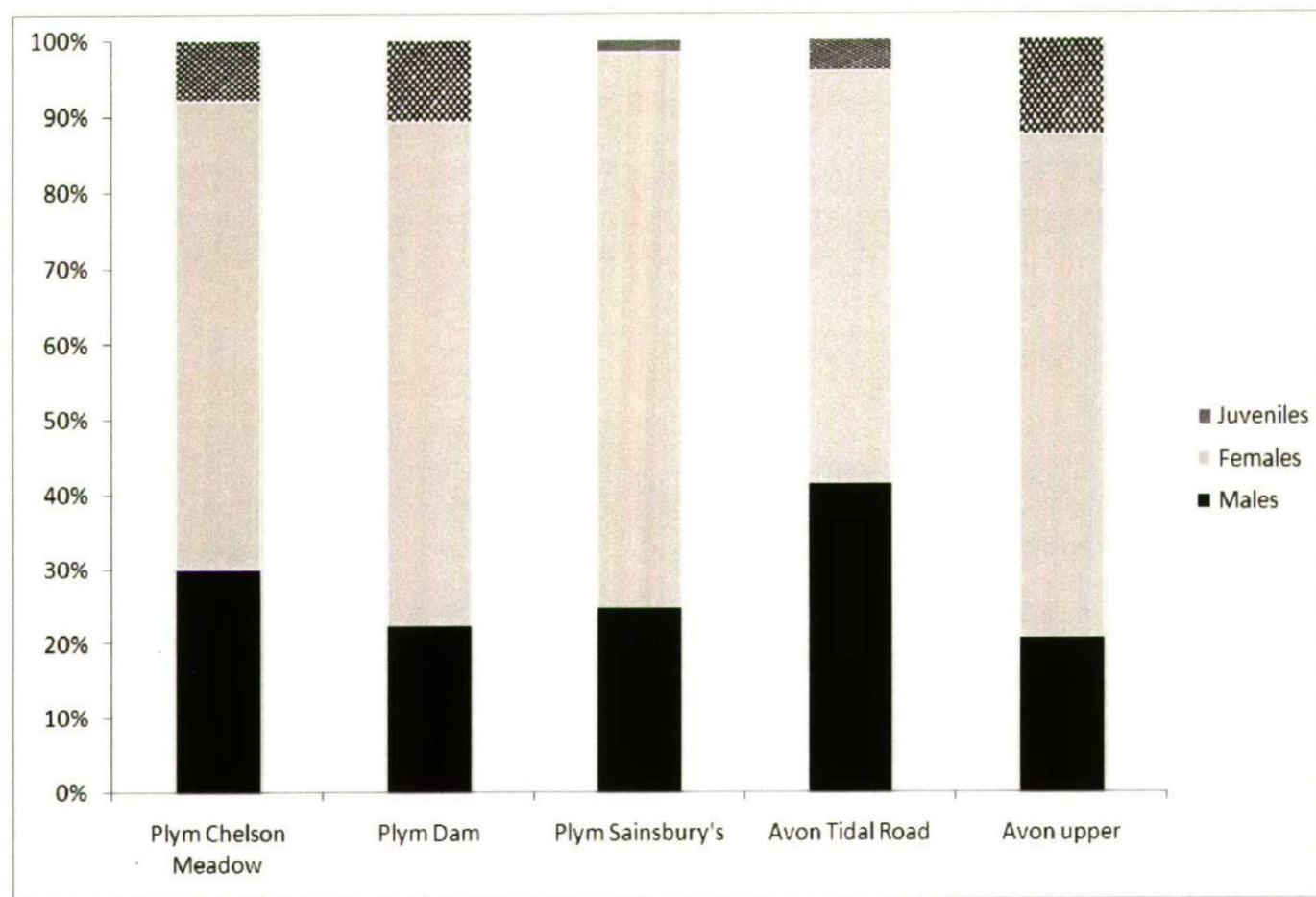


Figure 4.14: Mean proportional percentage abundance (ind./m²) of juvenile, female and male *G. chevreuxi* at different sites.

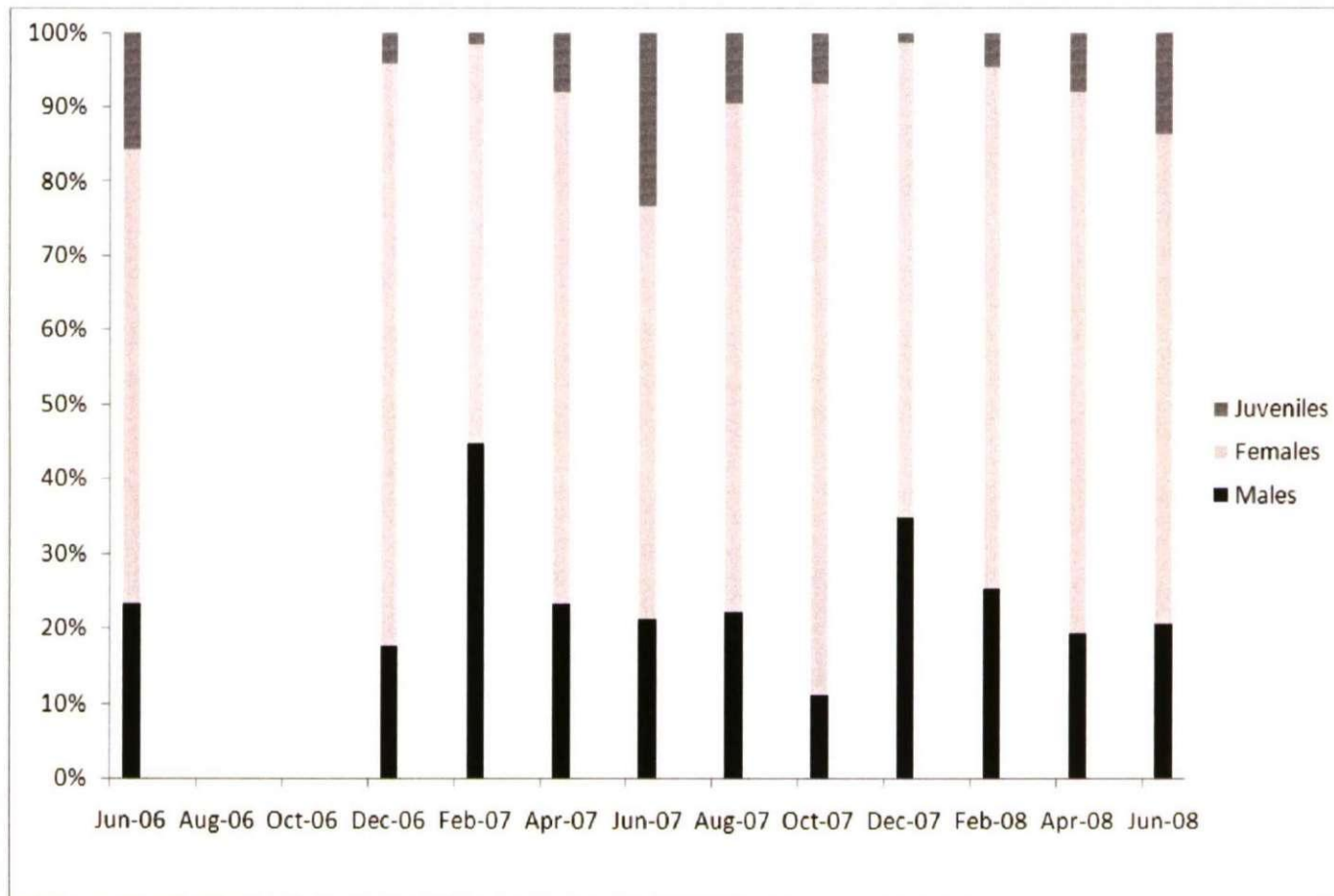


Figure 4.15: Mean proportional percentage abundance (ind./m²) of juvenile, female and male *G. chevreuxi* at different dates.

4.4 Discussion

Both spatial (site) and temporal (date) factors were found to affect the abundance of the test species. One sampling site for each species was shown to have much higher densities than all others. Densities varied widely for all species, with all sites empty in August 2006, and only *C. volutator* successfully collected in October 2006. There was no apparent reason for this, as sampling protocol was set from June 2006. Females dominated across all sites and dates sampled. For *C. voluator* the male:female ratio was 1:3.5, for *G. marinus* was 1:2.3 and for *G. chevreuxi* there were twice as many females as males (1:1.95). This biased ratio may indicate strong competition among males to fertilise multiple females (Ferreira et al. 2004, Bamber 1985), supported by previous observations that male *C. volutator* compete for access to receptive females (Forbes et al. 1996).

Table 4.11: Summary table of the partial η^2 and variance for all three amphipod species

	Significance	Partial η^2	Variance
<i>C. volutator</i>			
Site	<0.001	0.334	
Date	0.002	0.326	15.32%
Site x Date	<0.001	0.608	48.94%
<i>G. marinus</i>			
Site	0.013	0.254	
Date	<0.001	0.643	30.13%
Site x Date	<0.001	0.308	16.67%
<i>G. chevreuxi</i>			
Site	0.001	0.302	
Date	0.001	0.475	25.92%
Site x Date	<0.001	0.597	43.32%

From table 4.11, the results for *C. volutator* and *G. chevreuxi* were quite similar, with comparable partial η^2 . Date was a significant factor but the interaction between site

4.4. DISCUSSION

and date explained nearly half the variation. Yet, for *G. marinus*, site and date separately explained more of the abundance data, as the attributable variance for date was much higher and a larger partial η^2 than when site and date interacted. Overall, the effect each factor (site, date and their interaction) has on determining the density of each species can be ranked as follows:

1. Spatially - *C. volutator* > *G. chevreuxi* > *G. marinus*.
2. Temporally - *G. marinus* > *G. chevreuxi* > *C. volutator*.
3. Spatio-temporal - *C. volutator* > *G. chevreuxi* > *G. marinus*.

C. volutator The patchy nature of *C. volutator* has been noted previously at various spatial scales (Bergstrom et al. 2002). It is nevertheless necessary to address what is meant by the term 'patchiness'. Hildrew & Giller (1994) saw similarities between patch dynamics and metapopulation dynamics and noted that patchiness differs depending on the scale, biotic and abiotic factors considered. Hall et al. (1994) showed that patchiness can occur on small and large scales on intertidal sediments and that different processes can be operating at the different scales. Clumped distributions are the most frequently encountered and an examination of the variance of a series of samples can provide information on patch size (Hall et al. 1994). Thrush (1991) defined patchiness as abundances being autocorrelated at short distances. In the current study, the patterns of abundance were examined and related to the species distribution across space and time. The estimation of spatial patterning undertaken on the additional dataset from July 2007, confirmed the existence of the patterns found in the current analysis in that they were not an artifact of spatial autocorrelation, but were a true reflection of what was driving the abundance of these amphipod species.

4.4. DISCUSSION

The variability in the abundance data was partially explained by site differences. However, previous research by Hampel et al. (2009) notes spatial variability in the macrobenthos composition when comparisons were made between mud flat and salt marsh habitat in the large Schelde estuary (Netherlands and Belgium). Thus, there will be inherent variability in the current study when comparing a site on the Avon estuary which is located in a side stream in soft mud, to the Taw estuary 'salt marsh' site which has very different sediment composition and vegetation (for sediment analysis, see Chapter 5). Previous work by Hughes & Gerdol (1997) and Ysebaert et al. (1993) found lower densities on open mudflats compared to creeks or semi-enclosed bays, characterised by their low dynamic nature (Ysebaert et al. 1993). The faster flow of currents experienced by *C. volutator* in these open areas was postulated to move them away from these sites (Hughes & Gerdol 1997). Unexpectedly, the highest average density of *C. volutator* in the current study was found at the Taw 'main channel' site. This site was not as frequently covered by the tide as the 'main channel' site on the Avon estuary, but was not expected to have the highest abundance at this site due to the influence of tidal flow (Hughes & Gerdol 1997). The much more isolated Taw 'upper' site had the next highest abundance, indicating that despite apparently ideal habitat on the Avon estuary, densities of these populations must be limited by some other factor.

There did not appear to be any clear seasonal pattern with respect to the abundance or sex ratios of *C. volutator*. Although, when the interaction between date and site was examined, it did explain half of the variation attributable to the random factor in density terms. However, the ratio of male to juveniles also had a strong temporal variability component. The density of males corresponded to a decrease in the density of juveniles, with a corresponding increase when male density decreased. *Corophium*

4.4. DISCUSSION

volutator is known to undertake mate searching behaviour (Forbes et al. 1996). This behaviour may explain an increase in the presence of males in samples, as males would be encountered more frequently when searching for a mate and therefore may be more readily sampled. This type of behaviour can expose males to an increase in predation, and this increased risk must be compensated by the reproductive benefit of mating with multiple females (Bamber 1985).

The months of higher abundance of juveniles (June 2006 and October 2007) which were followed by a decline in the following months, may indicate a bivoltine population structure and a seasonality to this pattern. Previous research showed *C. volutator* to have one (Segerstråle 1959, McLusky 1968) or two generations per year (Fish & Mills 1979) and not continuous reproduction. Yet, due to the lack of any clear pattern, it is possible that *C. volutator* undergoes continuous reproduction or that there was a possible sampling artifact (see Crewe et al. 2001). As the current sampling method was seen to work in another study (Harris & Musko 1999) it is unclear why no obvious temporal patterns emerged. However, female *C. volutator* in the current study were sexually mature at a minimum of 2 mm. This contrasts with Bergstrom et al. (2002) who found *C. volutator* to reach adulthood at 5 mm or Flach & Debruin (1993) who found females matured at 4.1 mm. The difference in size of the populations could also be related to predation. Previous research on shorebird predation by Peer et al. (1986) explored further by Hilton et al. (2002), suggested that adult *C. volutator* off the east coast of Canada were reaching sexual maturity at a smaller size in order to avoid predation. Other research by Schneider et al. (1994) found a sex ratio bias towards female *C. volutator* that was not explained by predation, but by the predominance of female juveniles which might indicate asexual reproduction. Research on the

4.4. DISCUSSION

impact of conch (*Strombus gigas* (Linnaeus)) densities on predation by xanthid (*Micropanope* sp.) crabs showed that the proportional mortality decreased with increased density (Ray-Culp et al. 1999). The increased density in the patches of *C. volutator* may therefore proportionally reduce the chance of predation by birds. Further work on these populations of *C. volutator* needs to be conducted with regard to predation and the impacts this can have on the abundance-distribution relationship.

G. marinus Although site was found to be a significant factor, the interaction between site and date did not explain any more of the variation. Hence, site is an important factor, but it is not a simple relationship. Yu et al. (2003) found that the distribution of benthic amphipods on sandy shores was determined by the availability of prey species. This driver of distribution could also be occurring in the current study; Dick et al. (2005) found *G. (Echinogammarus) marinus* to be an active predator irrespective of habitat heterogeneity, with female gut contents indicating high rates of predation on the isopod *Jaera nordmanni* (Rathke). Therefore, the importance of site as a determinant of density might be related to the availability of prey rather than the site itself. Although not quantified in this study, a large number of the common estuarine isopod *Cyathura carinata* (Kroyer) (Ferreira et al. 2004) were frequently encountered in the samples, particularly from the Avon estuary, indicating a possible food source for *G. marinus* (author's own observation).

Habitat heterogeneity can also be an important factor determining predator-prey relationships. Prey capture of macroinvertebrates by the subtropical fish *Moenkhauisia sanctaefilomenae* was increased at the edge of floating macrophytes (*Eichhornia azurea* (Kunth)) (Etilé et al. 2009). Alga cover has been seen to influence the presence of amphipods in a *Caulerpa prolifera* (Forsskal) meadow in Spain, independent of site

4.4. DISCUSSION

or time of year (Sánchez-Moyano et al. 2007). *Gammarus marinus* is known to associate with patches of *F. vesiculosus* (Maranhao et al. 2001), however the amount of *Fucus* cover was not determined in the present study. Observation of the sampling sites reflects the results found here, the highest abundance of *G. marinus* was found at the Plym 'Chelson Meadow' site which had the highest density of *F. vesiculosus* (author's own observation). Hence, future work should consider the amount of available habitat when considering the distribution of *G. marinus*.

Research comparing both eutrophic and oligotrophic sub-estuaries showed a shift in trophic food levels by amphipods due to the reduced availability of prey (Fox et al. 2009). This should be considered for *G. marinus* specifically, due to its likely predatory nature (Dick et al. 2005). The Plym is a polluted estuary (Bard et al. 2008), and although this does not appear to affect the abundance of *G. marinus* negatively (see fig. 4.5), it could have consequences for its survival as a shift from carnivorous feeding owing to a lack of prey, sensitive to contamination, to omnivory can greatly increase the risk of predation due to increased exposure (Kolar & Rahel 1993). A small number of juveniles were present throughout the year in the two estuaries studied, with a possible seasonal pattern in male abundance, peaking during February of both years, without corresponding peaks in juveniles. This would point towards likely continuous reproduction, which has been found in *G. (Echinogammarus) marinus* in Portugal (Maranhao et al. 2001). Other studies showed that *G. marinus* has 3 to 4 broods per year indicating a multivoltine life cycle (Maranhao & Marques 2003); it is likely that such a pattern would not be discriminated by the sampling conducted in the present study.

4.4. DISCUSSION

G. chevreuxi The importance of both freshwater (MacNeil et al. 1997, Kelly et al. 2002) and marine (Yu & Suh 2002) amphipods as links in trophic structuring of a community has been studied. *Gammarus chevreuxi* has been found in both brackish conditions (Cunha & Moreira 1995) or close to fresh-water (Dieleman 1978, Subida et al. 2005) environments. In the current study, *G. chevreuxi* prevailed in rocky sediment in flowing streams and outflows with predominantly low salinities (1 - 5 ppt). This species differs from true freshwater species which inhabit lakes and streams, as these habitats are much more isolated and fragmented than the estuarine environment (Blackburn et al. 2006). However, this view of estuarine habitats is debated, as Bilton et al. (2002) describe these environments as discrete habitats defined by physiological limits. Therefore, estuaries present a challenge, as they can be viewed as both isolated and dynamic environments.

There was a possible seasonal trend present in the abundance data of *G. chevreuxi*, with a single breeding period. June in all three years showed the highest abundance of juveniles, with a slow decline, where animals presumably grew into male and female size classes. The overall abundance of these populations did vary widely with no animals found in August or October 2006, up to 3600 ind./m² in August 2007. This type of variation has been seen previously (Subida et al. 2005). It is possible that the juveniles are underrepresented by the chosen sampling method (e.g. Girisch & Dennert 1975). However, despite the same sampling mesh sizes used, the proportion of juveniles was much greater in the *G. chevreuxi* population in Portugal (Subida et al. 2005, Cunha & Moreira 1995) compared to the current study. The overall abundance of the population was similar to other species of *Gammarus* in Portugal (Marques & Nogueira 1991). Previous research has shown that abundance of *G. chevreuxi* was tied

4.4. DISCUSSION

to environmental variables particularly salinity (Subida et al. 2005, Dieleman 1978, Cunha & Moreira 1995). The lower abundance seen in this study may be related to the higher salinities which can be experienced in the Avon and Plym estuaries, but this would require further study.

Gammarus chevreuxi was found to be smaller at sexual maturity than in previous research (Barnes et al. 1979, Girisch et al. 1974). Females in other research were seen to brood eggs only above 4 mm, whereas in the current study, females were found with eggs from 2.7 mm. The population of *G. chevreuxi* in the Avon estuary was also found to have larger individuals of up to 12.527 mm whereas the largest in the Plym estuary was 10.981 mm; both of these were males. Barnes et al. (1979) found that the population of *G. chevreuxi* in a lagoon in Falmouth to be smaller (no individual larger than 10 mm) than those in Plymouth (Sexton 1924) even when reared in the laboratory, suggesting that phenotypic plasticity was not occurring and the population in Falmouth is distinct from the one in Plymouth. Hence, population differences found previously by Barnes et al. (1979), could also be currently occurring in the Avon and Plym estuaries.

From the current study, there does not appear to be any clear mechanism driving the relationships between abundance and distribution for these three species of amphipods. The interaction between temporal and spatial patterns were found to explain more of the attributable variation for *C. volutator* and *G. chevreuxi*, with only temporal variation explaining a large proportion for *G. marinus* (see table 4.11). Environmental factors such as salinity and temperature may be acting in conjunction with predation, habitat use and food availability. The lack of clear ecological mechanisms point towards the possibility of using the model-centric view of science proposed by del Rio (2008). This could help to explain and predict the drivers of these amphipod distribu-

Chapter 5

Abundance and occurrence in estuarine amphipods: A conceptual model

5.1 Introduction

There are many potential mechanisms underlying the distribution of animals within a habitat. In the previous chapters, salinity, temperature, spatial and temporal variation have been shown to affect species distribution and abundance (Chapter 4). The habitats experienced by the species vary widely from site to site, and these variations differ themselves depending on whether the microhabitat, the local or the regional geographic scale is considered. However, from a general viewpoint, three basic habitat templates can be conceptualised; *r*, *K* and *A* selection (Sainte-Marie 1991). *K*-selection habitats are stable, productive and rarely disrupted. *A*-selection habitats have low production but are stable, and *r*-selecting habitats are highly dynamic (Greenslade 1983). Brackish waters are hypothesised to be *r*-selecting (Southwood 1988, Sainte-Marie 1991), which is consistent with animals within these habitats being early to mature with high fecundity but short lifespan (Greenslade 1983).

In order to elucidate the mechanisms which drive the distribution of animals, models can be used to predict species' occurrences using correlations between occupancy patterns and environmental data (Meynard & Quinn 2007). Models represent a way to

5.1. INTRODUCTION

conceptualise and analyse datasets and can provide insight into an ecosystem which would not otherwise be available. Models are useful tools, particularly for viewing questions in a 'macro' ecological and physiological perspective. For example, in the 1990s the application of Artificial Neural Networks (ANN) emerged as a tool in biology (Lek & Guegan 1999). ANNs can be used in ecological situations that are highly complex. Input values are differently weighted and if the output data are incorrect, then the weighting can be altered (in an iterative fashion) until the correct output is generated. The ANN can then predict what the new output will be, given basic input data (Lek & Guegan 1999). Other computer based platforms have continued to make advancements in ecological modelling. For example, the predictions made by models can be integrated with Geographic Information Systems (GIS) to aid with management decisions regarding community conservation (Olden et al. 2006, Joy & Death 2004). Kearney & Porter (2009) reviewed models based in GIS and examined the value of both mechanistic and statistical models. Statistical models are correlative, linking species distribution with spatial data, whereas mechanistic models try to incorporate environmental variables and functional traits (Kearney & Porter 2009).

However far advanced computer modelling is developed, the origins of ecological modelling still have population dynamics at their core. For example, Freckleton et al. (2005) developed a model for the relationship between patch occupancy and local abundance for British birds by parameterising equations for each of 5 ecologically important processes; population increase, mean overall population size, potential incidence and mean local population size, extinction rates and colonisation rates. In this case they tested their model using range size and abundance data as well as occupancy and density measures of British bird species. Another example of statistic-based mod-

5.1. INTRODUCTION

elling centered on the amphipod *Talitrus saltator* (Montagu) (Anastacio et al. 2003). The model output was designed to match the recorded abundance over time and were based on age classes as these amphipods were known to reproduce three times a year. The models included; life span, abundance, recruitment, growth, sex ratios, age at maturation, and mortality (Anastacio et al. 2003). The output of the models were shown graphically as recruitment of individuals over time. Three of the models were found to have a large amount of variation compared to the test data and were rejected. The need to incorporate real time environmental conditions, such as the lack of recruitment during high summer temperatures, was shown to be important for the accuracy of the predictions made (Anastacio et al. 2003). Hence, models which are demographically dynamic are more accurate and, as seen in the various models present here, there are many different approaches available. Choosing the appropriate model will depend on the available data and expertise. In this study, a statistical approach was taken to parameterise elements within a conceptual framework, integrating physico-chemical parameters and physiological limit data with a variety of population demographic data.

Apart from choosing a model type, a further factor which requires careful consideration is choosing the appropriate animal. Most models have been based on terrestrial datasets, due to the availability of information (Meynard & Quinn 2007). However, using tractable marine animals such as amphipods, which live in a variable environment such as an estuary can pose different problems to those encountered on land. The environment is particularly variable; animals in the intertidal environment do not live in a strictly terrestrial or aquatic existence (Kearney & Porter 2009). They experience the convection and heat loss associated with living in the water compounded by exposure to the extremes in air temperature at low tide. Further to this, the temperature

5.1. INTRODUCTION

at which an animal can survive does not necessarily mean the population can persist there. Species have been found to be distributed among habitats which are well within their physiological limits (e.g. Gaston & Spicer 2001), while others are vulnerable to climate change by living at their thermal maximum (e.g. Stillman & Somero 2000, Stillman 2003). Estuarine animals also experience great variability in salinity regime, depending on the amount of tidal influence and the seasonality of freshwater input (e.g. Hampel et al. 2009).

Reproduction and survival are the most important processes for a population to persist (Gaston & Blackburn 2000, Kearney & Porter 2009). Within the same habitat there are often different mating strategies present. For example, *Gammarus marinus* and *G. chevreuxi* are both mate-guarders that attend to the female by attachment (Conlan 1991). Meanwhile, *Corophium volutator* is also considered to be a mate-guarder which attends to females, but does so not by attachment as in *Gammarus* (Conlan 1991) but by remaining in the burrow to defend the female against other males (Forbes et al. 1996). The number of broods produced can also vary widely (Conlan 1991). *Gammarus marinus* has been found to have as many as three broods while *G. chevreuxi* has one (Conlan 1991) but this species has also been seen to breed continuously (Sexton 1928, Subida et al. 2005). Brood number is also variable in *C. volutator*. Some populations produce one generation per year (Segerstråle 1959, McLusky 1968), others two (Peer et al. 1986) and yet others as many as 4 broods per year (Muus 1967).

Other factors can also impact the distribution of animals within the habitat. For example, both salinity and temperature were shown to affect brood size which was also dependent on the size of female *G. zaddachi* (Sexton) (Kinne 1960). Research undertaken by Meadows (1964b) found that a differential distribution of body size in *C. volutator*

5.1. INTRODUCTION

existed due to larger individuals preferring deeper mud. Bergstrom's (2002) analysis found that the patchiness which is characteristic of *C. volutator* can be attributed to the aggregations of juveniles in their maternal burrows. All of these various factors can affect the abundance and distribution of such amphipod species. These factors can, however, be quantified in various ways and can be used in a model to validate whether or not they are driving the abundance and distribution patterns noted previously. All the interactions which can be affecting the abundance-occupancy relationship need to be considered (see fig. 5.1).

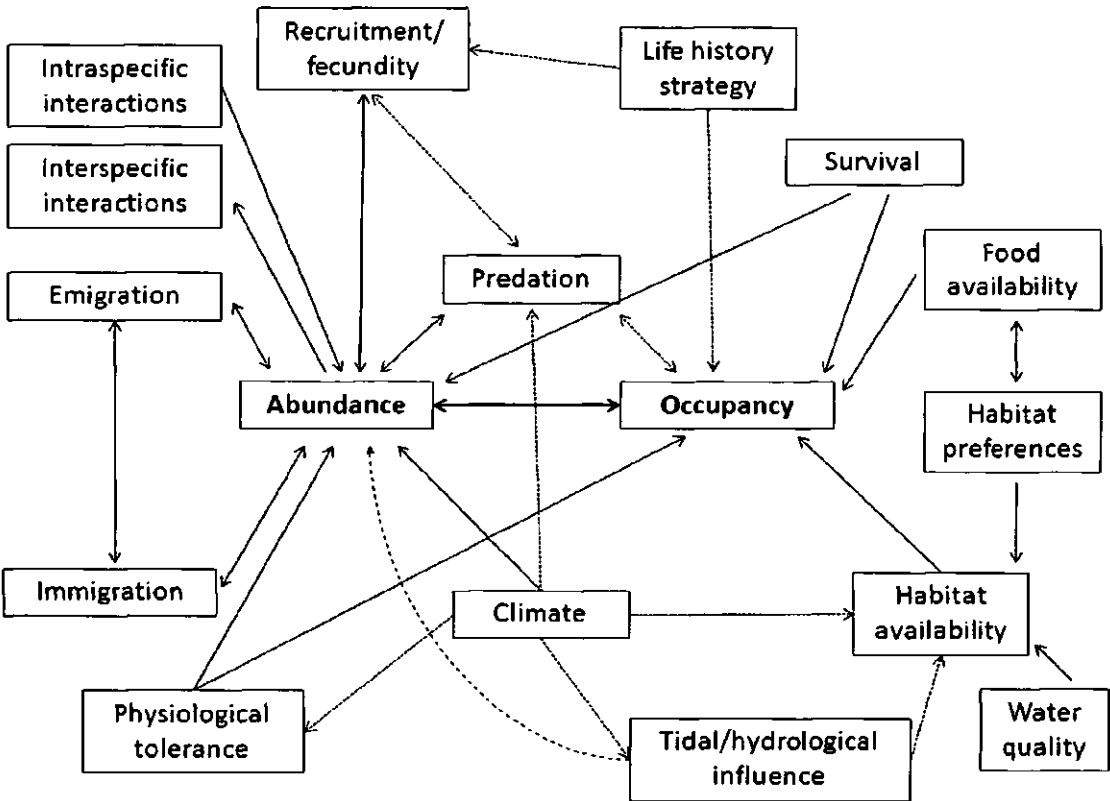


Figure 5.1: Possible factors affecting the abundance-occupancy relationship.

As it is not possible to incorporate all of the above factors, the aim of this chapter was to develop a conceptual model and to use it as a tool to predict the presence and absence

of amphipod species with available data. The model was constructed using physiological (Chapter 2 and 3) and spatio-temporal data collected previously (Chapter 4), together with additional data taken from the literature for the three amphipod species: *C. volutator*, *G. marinus* and *G. chevreuxi*. A variety of biotic factors and abiotic factors were considered. Density, occupancy, sex ratios, juvenile to adult ratios and site and physiological preferences were used as factors to model the presence/absence data collected.

5.2 Material and Methods

5.2.1 Model factors

Two factors formed the basis of the model from the ecological dataset from the Avon, Plym, Taw and Torridge estuaries. The response variable was presence/absence from August 2006 to June 2008, with species and site as fixed nominal predictors. Other elements of the model included:

Population demographics Multiple factors were derived from the population composition data.

1. Mean sample density - Ind./m² from June 2006 to April 2008, a continuous predictor used as a scale predictor.
2. Occupancy - Proportion of samples occupied from June 2006 to April 2008, ordinal predictor.
3. Fecundity - Average egg load multiplied by number of ovigerous females in the population, scale predictor.

5.2. MATERIAL AND METHODS

Average egg load per female was derived from the literature. For *C. volutator*, females on average carried 22 eggs (10.4-30 eggs) (Peer et al. 1986, Fish & Mills 1979, Hilton et al. 2002). For *G. marinus* females carried on average 34.9 eggs (14.8-55 eggs) (Marques & Nogueira 1991). Finally, *G. chevreuxi* was found to have on average 8 eggs (up to 32 eggs) (Subida et al. 2005). In the current study, ovigerous females comprised on average 3.5% (0 - 24%) of *C. volutator* collected, 9.6% (0-43%) of *G. marinus* collected and 12% (0-36%) of *G. chevreuxi* collected.

Climate variables Climatic variables were derived from local weather station data. For the Taw/Torridge estuary, data from the Chivenor (Barnstable) station were used, and data for the Avon and the Plym estuaries were taken from the Plymouth station (MetOffice 2009). All weather data were used as scale predictors in the model. To ascertain whether or not the previous month's weather was affecting the presence/absence of these amphipods, 'lagged' data was also incorporated into the model. 'Lagged' refers to weather data from the month before the sampling took place. This one month lag was chosen after preliminary examination of the data; patterns were noted in the mean abundance with the current and previous months' climate variables.

1. Rainfall - Obtained from weather stations from August 2006 to June 2008.
2. Temperature range - Maximal subtracted from minimal temperature taken from weather stations from August 2006 to June 2008.
3. Mean, maximal and minimal temperatures - Taken from weather stations from August 2006 to June 2008.
4. Lagged rainfall, temperature range, mean, maximal and minimal temperatures - Obtained from weather stations from July 2006 to May 2008.

5.2. MATERIAL AND METHODS

Preferences To incorporate physiological and site variation, preferences were derived from suitability of environmental factors.

1. Physiological preference - ordinal predictor.
2. Site preference - ordinal predictor.

For physiological tolerances, using the MO_2 (Chapter 2) and CTMax and CTMin data (Chapter 3) collected previously, as well as the salinity and temperature data collected across seasons at each site, each species can be ranked according to what temperature range it was able to tolerate and how this was modified by exposure to salinity and if this changed temporally across seasons. *Corophium volutator* and *G. marinus* ranged between 2.5 and 4, and *G. chevreuxi* ranged between 3 and 4 (see table 5.1).

Site preferences for *C. volutator* can be derived from the availability of suitable muddy sediment and amount of tidal flow and ranged between 3 and 5 (out of 5). For *G. marinus*, significant coverage of the site by *Fucus vesiculosus* ranked the site higher than those with less algae cover. For *G. chevreuxi*, a high amount of rocky cobbles and a large amount of water flow ranked the site higher than those with less water flow with muddy sediment dominating. The sites for *G. marinus* were found to be between 3 and 5 (out of 5), and *G. chevreuxi* were between 2 and 5 (out of 5) (see table 5.1).

The rankings were derived from familiarity with the sites, salinity and temperature readings taken throughout the sampling period along with knowledge of the optimal physiological limits derived from the laboratory experimentation. For example, habitat availability for *G. marinus* was ranked as 5 at the 'Chelson Meadow' site which has 100% *Fucus* cover, while a site ('Dam') with less than 20% available cover was ranked 1.

Table 5.1: Rankings (low = 1, high = 5) of each species and site combination for both physiological and site preferences.

	Salinity range	Temperature range	Season	Average	Habitat availability	Sediment or water flow	Average
<i>C. volutator</i>							
Avon Tidal Rd	3	2.5	2	2.5	4	4	4
Avon main channel	3	3	3	3	3	3	3
Avon upper stream	3	2	2.5	2.5	5	3	4
Taw salt marsh	5	3	4	4	4	4	4
Taw main channel	4	5	3	4	4	4	3
Taw upper stream	4	2.5	4	3.5	5	5	5
Torrige	2	3	4	3	5	3	4
<i>G. marinus</i>							
Avon Hexdown	3	5	4	4	3	3	3
Avon tidal Rd	3	5	4	4	2	4	3
Plym Dam	3	5	4	4	1	3	2
Plym Chelson meadow	1	5	4	2.5	5	5	5
<i>G. chevreuxi</i>							
Avon Tidal Rd	2	3	4	3	4	4	4
Avon upper stream	3	3	3	3	4	4	4
Plym Dam	3	5	4	4	5	5	5
Plym Chelson meadow	4	1	4	3	4	2	3
Plym upper stream	3	3	3	3	2	2	2

5.2. MATERIAL AND METHODS

Sediment analysis An additional dataset was available for *C. volutator* from the length of the Avon estuary. The data were collected using 10 x 10 cm cores, with 80 samples collected in July 2007. A BioEnv analysis (Clarke & Ainsworth 1993) of this data indicated the distribution of *C. volutator* was affected by the sediment type (β coefficient = 0.69, odds ratio = 1.28, $P < 0.001$). The same sediment analysis was conducted on samples from the current study sites on the Avon, Taw and Torridge estuaries, per seasons (2 summers and 2 winters).

The sediment grain size was determined using a Malvern Mastersizer 2000 laser particle-sizer. This allowed for additional factors to be included in the models for *C. volutator*.

1. Mean ϕ - scale predictor.
2. Mean geometric grain size - scale predictor.
3. Mean arithmetic grain size - scale predictor.

5.2.2 Binary Logistic Regression

The model was constructed following a stepwise process (see fig. 5.2).

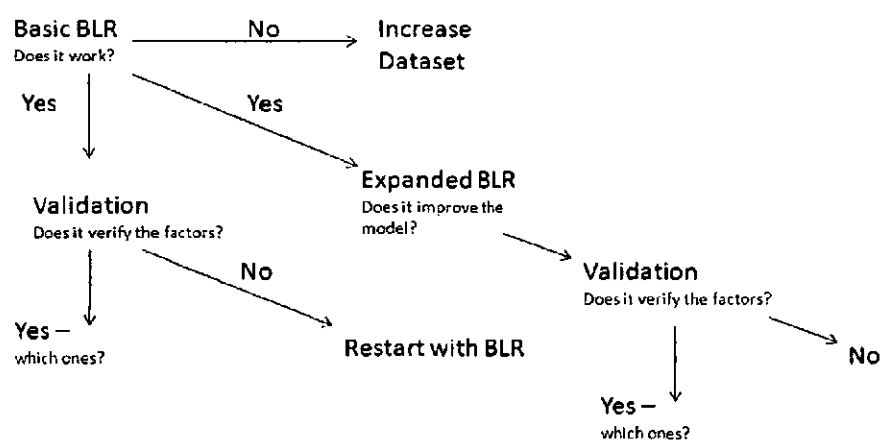


Figure 5.2: Flow chart for the model process, for both the overall dataset and the individual species models.

Basic BLR

To determine if the model was functional, a basic Binary Logistic Regression (BLR) was undertaken as the first step towards the complete model (see table 5.2). Once the initial BLR was found to work, further data could be added to the models to improve the accuracy.

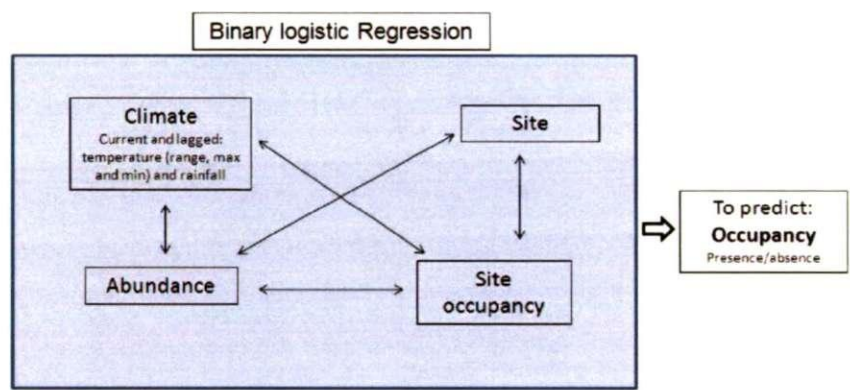


Figure 5.3: Pictographic representation of the binary logistic regression.

The available data (population composition, weather data, site and physiological preferences) were used in a BLR, conducted in SPSS ver.16.0 (SPSS Inc. Chicago) (see table 5.2). BLR was chosen because this type of analysis used a stepwise model, with each factor being added into the equation and the final model step (between 1 and 8 steps) was composed of the significant factors in order of importance. The output of the model was compared to actual presence/absence data (see fig. 5.3), with the results being presented as an R^2 value followed by a percentage of correct presence/absence predictions (1 and 0) for the best model selected (e.g. R^2 a, b%).

Expanded BLR

Fecundity, site preference and physiological preferences were used as additional factors in each of the 8 model permutations as above (see fig. 5.4 and table 5.2).

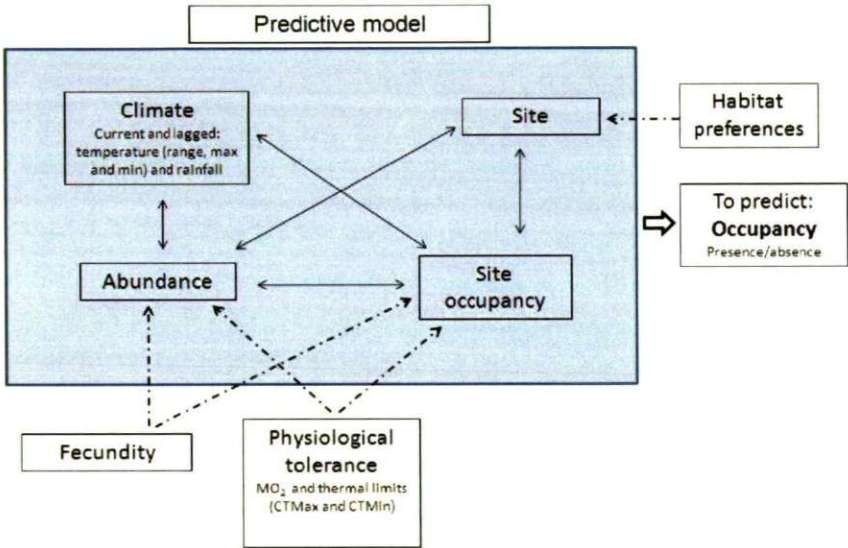


Figure 5.4: Pictographic representation of the expanded statistical model.

Table 5.2: The 8 model permutations for the basic BLR and the expanded BLR

	Models								Basic BLR	Expanded BLR
	1	2	3	4	5	6	7	8		
Species	x	x	x	x	x	x	x	x		
Site	x	x	x	x	x	x	x	x		
Occupancy	x	x	x	x	x	x	x	x		
Density	x	x	x	x	x	x	x	x		
Rainfall	x	x			x			x		
Temperature range	x		x							
Lagged rainfall			x	x		x		x		
Lagged temp range		x		x						
Mean temperature					x	x				
Max temperature					x	x				
Min temperature					x	x				
Lagged mean temperature								x	x	
Lagged max temperature								x	x	
Lagged min temperature								x	x	
Fecundity	x	x	x	x	x	x	x	x		
Physiological preferences	x	x	x	x	x	x	x	x		
Site preferences	x	x	x	x	x	x	x	x		

Validation

A common way to validate models is to split the data, with between 20 and 50% not used in the model construction, to verify the results (Meynard & Quinn 2007, Dedecker et al. 2007, Statzner et al. 2008). As shown in Chapter 4, there was a temporal component to the variation in abundance and distribution of the amphipod species. To validate the models, the data were split into two sets by years. The first dataset, therefore spanned from June 2006 to June 2007 and the second from August 2007 to June 2008. If the models included the same significant factors as before they were split, this would indicate that the model was robust, as only factors which were significant across time would be included.

5.2.3 Single species models

Basic BLR

Following the general modelling, each species (*C. volutator*, *G. marinus* and *G. chevreuxi*) was modelled separately with the same 8 model combinations as the basic BLR using all of the factors with the exclusion of 'species'.

Expanded BLR

The same additional factors (physiology, site preference and fecundity) were added to the datasets for each of the three species.

Further to this, the sediment analysis conducted on sites where *C. volutator* was collected, meant that had three additional factors could be added to the model (mean ϕ , mean geometric grain size and mean arithmetic grain size).

5.3. RESULTS

The BLR was run in the same manner as previously stated for each species individually.

Validation

To verify the single species models, the same approach was taken with each species individually. If the significant factors remained the same in both time series, then the model for that species was considered robust.

5.3 Results

5.3.1 Whole dataset models

All species

Basic BLR There were seven significant factors in the model with the highest R^2 value (R^2 0.376, 72.7%) (see table 5.3). However, not all these factors were validated in subsequent models (see summary table 5.6).

Table 5.3: Results of the basic BLR including all species, where B is the coefficient for the constant and exp(B) is the odds ratio.

Factor	B	DF	Significance	Exp (B)
Density	0.001	1	0.009	1.001
Occurance	0.846	1	0.001	2.329
Species	0.571	2	0.000	2.188
Site	1.550	10	0.000	5.653
Rain (lagged)	0.023	1	0.000	1.023
Mean	0.746	1	0.000	2.108
Min	-0.872	1	0.000	0.418
Constant	-5.562	1	0.000	0.004

Expanded BLR The additional factors did not change the model and the significant factors remained the same.

5.3. RESULTS

Validation The only factors which remained consistently significant throughout all the tests were site and species (see tables 5.4 and 5.5). The validation of the basic BLR using June 2006 to June 2007 only added lagged minimal temperature (R^2 0.426, 79.8%), whereas the August 2007 to June 2008 validation included density and lagged rainfall as significant factors (R^2 0.385, 77.3%). More factors were found to be significant when the expanded BLR model was validated, but the R^2 changed very little (see summary table 5.6).

Table 5.4: Results of the validation for the basic BLR model including all species, where B is the coefficient for the constant and exp(B) is the odds ratio.

Factor	B		DF		Significance		Exp (B)	
	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008
Density		0.001		1		0.099		1.001
Site	2.277	1.933	10	10	<0.001	<0.001	14.445	11.968
Species	0.810	0.646	2	2	<0.001	<0.001	3.194	2.600
Lagged rain		0.039		1		<0.001		1.040
Lagged minimum	-0.323		1		<0.001		0.724	
Constant	-0.383	-5.504	1	1	0.557	<0.001	0.682	0.004

Table 5.5: Results of the validation for the expanded BLR model including all species, where B is the coefficient for the constant and exp(B) is the odds ratio.

Factor	B		DF		Significance		Exp (B)	
	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008
Density		0.001		1		0.099		1.001
Site	1.964	1.933	10	10	<0.001	<0.001	16.806	11.968
Species	0.861	0.646	2	2	<0.001	<0.001	5.252	2.600
Lagged rain		0.039		1		<0.001		1.040
Lagged minimum	-0.336		1		<0.001		0.715	
Fecundity	0.006		1		0.001		1.006	
Physiology	1.409		1		0.032		4.092	
Constant	-5.126	-5.504	1	1	0.031	<0.001	0.006	0.004

Table 5.6: Summary table for all models including all species, with R^2 values and accuracy of predicted presence/absence for each BLR model

	Basic BLR	Validation		Additional factors BLR	Validation	
		June 06 - June 07	Aug 07 - June 08		June 06 - June 07	Aug 07 - June 08
		R^2 0.376, 72.7%	R^2 0.426, 79.8%		R^2 0.46, 79.6%	R^2 0.385, 77.3%
Species	6	3	2	6	3	3
Site	3	2	3	3	2	2
Occupancy	1			1		
Density	7		4	7		4
Rainfall						
Temperature range						
Lagged rainfall	2		1	2		1
Lagged temp range						
Mean temperature	5			5		
Max temperature						
Min temperature	4			4		
Lagged mean temperature						
Lagged max temperature						
Lagged min temperature		1			1	
Physiological preferences					5	
Site preferences						
Fecundity					4	

5.3. RESULTS

5.3.2 Single species models

C. volutator

Basic BLR There were four significant factors in the best fit model for *C. volutator* (see table 5.7 and summary table 5.10).

Table 5.7: Results of the basic BLR for *C. volutator*, where B is the coefficient for the constant and exp(B) is the odds ratio.

Factor	B	DF	Significance	Exp (B)
Density	0.001	1	0.013	1.001
Rain (lagged)	0.013	1	0.001	1.013
Site	1.615	6	0.000	7.035
Max (lagged)	-0.196	1	0.000	0.822
Constant	0.860	1	0.203	2.362

Expanded BLR The results of the sediment analysis can be found in table 5.8. The model for *C. volutator* was not altered by the inclusion of additional data.

Table 5.8: Results of the sediment analysis for three different grain size parameters

		Average	Minimum	Maximum
Mean ϕ	Winter 2007	4.25	2.76	5.95
	Summer 2007	4.18	2.18	6.17
	Winter 2008	5.05	4.45	5.68
	Summer 2008	4.42	3.14	6.01
Mean geometric grain size	Winter 2007	60.04	16.50	118.57
	Summer 2007	71.55	14.40	187.76
	Winter 2008	31.53	19.77	44.99
	Summer 2008	55.17	15.80	105.11
Mean arithmetic grain size	Winter 2007	107.24	38.58	173.35
	Summer 2007	124.48	37.70	288.30
	Winter 2008	70.06	42.87	90.98
	Summer 2008	99.41	37.33	163.40

Validation The models were altered when the data was split for validation (see tables 5.8 - 5.10). None of the factors remained consistently significant throughout the modelling process.

5.3. RESULTS

Table 5.9: Results of the validation for the basic BLR model for *C. volutator*, where B is the coefficient for the constant and exp(B) is the odds ratio.

Factor	B		DF		Significance		Exp (B)	
	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008
Occupancy	-1.526		1		0.007		0.217	
Site	2.754	2.256	6	6	<0.001	<0.001	23.779	18.705
Rainfall		-0.024		1		0.001		0.976
Lagged maximum	-0.361	-0.818	1	1	<0.001	<0.001	0.697	0.441
Lagged minimum		0.630		1		0.012		1.877
Constant	4.407	6.302	1	1	<0.001	<0.001	82.007	545.627

Table 5.10: Results of the validation for the expanded BLR model for *C. volutator*, where B is the coefficient for the constant and exp(B) is the odds ratio.

Factor	B		DF		Significance		Exp (B)	
	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008
Site		0.854		6		0.001		6.499
Lagged rainfall	0.015		1		0.028		1.015	
Lagged maximum	-0.754	-0.906	1	1	<0.001	<0.001	0.471	0.404
Lagged minimum	0.769	0.869	1	1	<0.001	0.001	2.157	2.384
Mean ø	-3.706	13.461	1	1	<0.001	<0.001	0.025	701518.8
Mean geometric	-0.062		1		<0.001		0.940	
Mean arithmetic		0.289		1		<0.001		1.335
Constant	23.939	-83.469	1	1	<0.001	<0.001	2.492E10	0.000

The addition of the sediment analysis data altered the results of the model, and demonstrates the importance of site sediment composition. However, no single factor was significant in all models tested.

Table 5.11: Summary table for all models for *C. volutator*, with R^2 values and accuracy of predicted presence/absence for each BLR model.

	Basic BLR	Validation		Additional factors BLR	Validation	
		June 06 - June 07	Aug 07 - June 08		June 06 - June 07	Aug 07 - June 08
	R^2 0.437, 75.7%	R^2 0.485, 80.5%	R^2 0.509, 85.9%	R^2 0.437, 75.7%	R^2 0.344, 82.5%	R^2 0.553, 83.9%
Site	2	2	1	2		1
Occupancy		3				
Density	4			4		
Rainfall			3			3
Temperature range						
Lagged rainfall	3			3	4	
Lagged temp range						2
Mean temperature						
Max temperature	1			1		
Min temperature						
Lagged mean temperature						
Lagged max temperature		1	2		1	
Lagged min temperature			4		4	
Physiological preferences						
Site preferences						
Fecundity						
Mean ϕ					2	4
Mean geometric grain size					3	
Mean arithmetic grain size						5

5.3. RESULTS

G. marinus

Basic BLR There were only two significant factors in the best fit model for *G. marinus* (see table 5.12). This model had the lowest R^2 of all the single species models (R^2 0.228, 71.2%), with only occupancy and lagged rainfall included as significant factors.

Table 5.12: Results of the basic BLR model for *G. marinus*, where B is the coefficient for the constant and exp(B) is the odds ratio.

Factor	B	DF	Significance	Exp (B)
Occupancy	2.074	1	0.000	7.954
Rain (lagged)	0.026	1	0.000	1.027
Constant	-3.629	1	0.000	0.027

Expanded BLR The additional factors did not change the significant factors for the model for *G. marinus*.

Validation Although the R^2 value increased with the splitting of the datasets for *G. marinus*, no single factor was consistently significant for all models (see tables 5.13 to 5.15).

Table 5.13: Results of the validation for the basic BLR model for *G. marinus*, where B is the coefficient for the constant and exp(B) is the odds ratio.

Factor	B		DF		Significance		Exp (B)	
	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008
Site		-0.480		3		0.012		0.792
Rainfall		0.017		1		0.020		1.017
Lagged rainfall	0.069		1		<0.001		1.071	
Lagged temp range		-0.665		1		0.005		0.514
Maximum	0.538		1		<0.001		1.713	
Constant	-15.799	3.584	1	1	<0.001	0.024	0.000	36.031

5.3. RESULTS

Table 5.14: Results of the validation for the expanded BLR model for *G. marinus*, where B is the coefficient for the constant and exp(B) is the odds ratio.

Factor	B		DF		Significance		Exp (B)	
	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008
Density	-0.075		1		0.003		0.928	
Site		-0.480		3		0.012		0.792
Rainfall		0.017		1		0.020		1.017
Lagged rainfall	0.058		1		<0.001		1.060	
Lagged temp range		-0.665		1		0.005		0.514
Maximum	0.418		1		0.002		1.519	
Fecundity	0.069		1		0.003		1.071	
Constant	-1.405	3.584	1	1	<0.001	0.024	0.000	36.031

Table 5.15: Summary table for all models for *G. marinus*, with R^2 values and accuracy of predicted presence/absence for each BLR model.

	Basic BLR	Validation		All factors BLR	Validation	
		June 06 - June 07	Aug 07 - June 08		June 06 - June 07	Aug 07 - June 08
	R^2 0.228, 71.2%	R^2 0.414, 88.3%	R^2 0.257, 72.5%	R^2 0.228, 71.2%	R^2 0.558, 92.5%	R^2 0.257, 72.5%
Site						
Occupancy	1	1	2	1		2
Density					2	
Rainfall			3			3
Temperature range						
Lagged rainfall	2	2		2	3	
Lagged temp range			1			1
Mean temperature						
Max temperature		3			4	
Min temperature						
Lagged mean temperature						
Lagged max temperature						
Lagged min temperature						
Physiological preferences						
Site preferences						
Fecundity					1	

5.3. RESULTS

G. chevreuxi

Basic BLR The best fit model for *G. chevreuxi* had 4 significant factors (R^2 0.462, 77.7%) (see table 5.16).

Expanded BLR The model changed with the additional factors, and seven factors were significant predictors (see table 5.16 and summary table 5.19).

Table 5.16: Results of the basic and expanded BLR models for *G. chevreuxi*, where B is the coefficient for the constant and exp(B) is the odds ratio.

Factor	B		DF		Significance		Exp (B)	
	Basic BLR	Exp BLR	Basic BLR	Exp BLR	Basic BLR	Exp BLR	Basic BLR	Exp BLR
Density		0.002		1		0.001		1.002
Occupancy	1.208	1.726	1	1	0.003	0.001	3.347	5.618
Rain (lagged)	0.043	0.034	1	1	<0.001	<0.001	1.044	1.035
Site	-1.202	-1.805	4	4	<0.001	<0.001	0.394	0.223
Temperature range	0.544		1		<0.001		1.722	
Mean		1.722		1		<0.001		5.597
Minimum		-2.016		1		<0.001		0.133
Fecundity		-0.010		1		<0.001		0.990
Constant	-7.106	-5.406	1	1	<0.001	<0.001	0.001	0.004

Validation The models were altered with the splitting of the data and with the additional data (see tables 5.17 and 5.18). Only occupancy remained as a consistently significant factor in all the model permutations and validations (see summary table 5.18).

Table 5.17: Results of the validation for the basic BLR model for *G. chevreuxi*, where B is the coefficient for the constant and exp(B) is the odds ratio.

Factor	B		DF		Significance		Exp (B)	
	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008
Density	0.012		1		0.045		1.012	
Occupancy		1.292		1		0.017		3.641
Site	-3.354	-0.499	4	4	0.004	0.002	0.052	0.424
Mean	165.654		1		0.021		8.76E71	
Maximum	-79.672		1		0.022		<0.001	
Minimum	-86.857		1		0.020		<0.001	
Lagged rainfall	0.049	0.049	1	1	<0.001	<0.001	1.050	1.050
Constant	-6.835	-4.382	1	1	0.064	<0.001	0.001	0.012

5.3. RESULTS

Table 5.18: Results of the validation for the expanded BLR model for *G. chevreuxi*, where B is the coefficient for the constant and exp(B) is the odds ratio.

Factor	B		DF		Significance		Exp (B)	
	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008
Density	0.057	0.002	1	1	<0.001	0.011	1.059	1.002
Occupancy		1.975		1		0.004		7.208
Site		-1.418		4		0.001		0.413
Lagged rain		0.069		1		<0.001		1.071
Mean	1.989		1		<0.001		7.310	
Minimum	-2.770		1		<0.001		0.063	
Fecundity	-0.093	-0.009	1	1	<0.001	0.001	0.912	0.991
Site preference	-0.850		1		0.003		0.427	
Constant	2.696	-5.509	1	1	0.066	0.001	14.815	0.004

Table 5.19: Summary table for all models for *G. chevreuxi*, with R^2 values and accuracy of predicted presence/absence for each BLR model.

	Basic BLR	Validation		All factors BLR	Validation	
		June 06 - June 07	Aug 07 - June 08		June 06 - June 07	Aug 07 - June 08
	R^2 0.462, 77.7%	R^2 0.666, 86%	R^2 0.431, 74%	R^2 0.547, 81%	R^2 0.603, 86.7%	R^2 0.504, 75.3%
Site	3		1	3		1
Occupancy	1	3	3	1	3	3
Density		1			1	5
Rainfall						
Temperature range	4					2
Lagged rainfall	2		2	2		
Lagged temp range						
Mean temperature		4		6	4	
Max temperature		5				
Min temperature		2		5	2	
Lagged mean temperature						
Lagged max temperature						
Lagged min temperature						
Physiological preferences						
Site preferences					6	
Fecundity				4	5	4

5.4 Discussion

The models were not found to be robust. Very few factors remained in the models throughout the validation process. The whole dataset models including all species were found to have site and species as consistent factors, which was perhaps to be expected. Only *G. chevreuxi* had a consistent factor throughout model validation, occupancy. The validation revealed that the year from June 2006 to June 2007 was unusual. For all single-species models, factors were found to be significant in all cases except during that time period. This temporal component in the models reinforces the results of Chapter 4, as the density across the sampling period differed significantly for all three amphipod species.

Previous work has shown that modelling can be viewed as a stepwise elimination process (Anastacio et al. 2003). If the basic BLR is disregarded as a step in the modelling process, and the first half of the dataset (June 2006 to June 2007) is set aside, more factors can be included in the expanded model. For the whole dataset expanded model; species, site, density and lagged rainfall were validated as model factors by the August 2007 to June 2008 dataset. For *C. volutator* and *G. marinus* occupancy becomes the significant factor in the model. In Chapter 4, density differed between sites for all species. Obviously, the temporal component was much stronger than the spatial component for the presence/absence of *C. volutator* and *G. marinus*.

It is not surprising that occupancy comes out as the most important overall model factor. This may be a reflection of the intra and interspecific abundance-occupancy relationship. Abundance-occupancy relationships are most commonly positive and frequently encountered over a wide number of spatial scales (Gaston 1996). This is

5.4. DISCUSSION

due to the inherent relationship in which locally abundant species have been found to occupy more sites than locally rare species (Gaston et al. 2000). In the current study, the abundance of three amphipod species was studied across several sites. This size of study would be considered a partial analysis, as only a proportion of the total geographic range of these species is covered (Gaston 1996). Nevertheless, this is still a valid approach given that most relationships are apparent across small and large spatial scales (Gaston 1996). Furthermore, Gaston & Curnutt (1998) primarily use occupancy as the dependent variable, with abundance as the independent variable. However, in the current study, these can be reversed as there is a causal relationship between the current presence/absence and the occupancy of sites during the next sampling date.

Gammarus chevreuxi was found to have the most additional factors in the model; site, occupancy and fecundity. Despite site being a significant factor on the distribution of all three species in the previous chapter, it was unexpected to find only *G. chevreuxi* with site as a significant factor in the model. *Gammarus chevreuxi* has been collected from a variety of habitats (Dieleman 1978, Girisch & Dennert 1975, Subida et al. 2005). Perhaps this is a reflection of the more stable micro habitats in the current study. The presence/absence of *G. chevreuxi* may be more consistent within sites compared to the more dynamic and variable environmental conditions experienced on the tidally exposed sites, where *C. volutator* and *G. marinus* were collected.

Therefore, despite the apparent weakness of the models developed here, when properly contextualised, the models can be viewed differently. The expanded BLR can be used to accurately predict presence/absence data, provided the dataset is interpreted in a temporally finite context.

All models make certain assumptions, whether it is the shape of the relationship be-

5.4. DISCUSSION

tween variables (e.g. linear, Gaussian or threshold) or that all environmental variables have equal impact on the distribution of species (Meynard & Quinn 2007). Olden et al. (2006) found very accurate presence/absence results for fish assemblage modelling using ANN. However, this work was conducted on data collected over a 20 year period. The environmental variability and its impact on species occurrence can be accurately predicted when month to month or year to year variability is reduced. In the current work, the two year dataset was shown to be insufficient to develop a model which is as robust as others. However, the reduction in the temporal variation can cause important events to be missed. The continued presence of a population at given site can be determined by a single event. Therefore, even though the model robustness is reduced by small time scale variations, ignoring this environmental variability can result in missing the cause of a species absence.

Chapter 6

Final Discussion

The overall aim of this thesis was to produce a predictive model for amphipod abundance and distribution. It was envisaged that provided with geographic information, physiological tolerance data could be inputted and the presence/absence could be predicted along with abundance estimates. The central question to this thesis was: can physiological limits be used to predict the presence or absence of amphipods given habitat information? Before it is possible to answer this question, each component will be examined.

Rate of oxygen consumption (MO_2)

The aim of Chapter 2 was to investigate the effect of biotic and abiotic factors on physiological capacity in the form of MO_2 of the three amphipod species. The objective was to measure the oxygen consumption of the three amphipod species over different lengths of exposure to test for acute (4 hour) and acclimatory (5 days) responses to changes in salinity. The central question was: does length of exposure to a change in salinity alter the metabolic oxygen demand of amphipods? The answer is yes, however, the three amphipod species differed in their acclimatory ability and salinity tolerances. The different time scales were critical to further the understanding of how these amphipod species cope physiologically with the highly variable salinity experienced in estuaries. *Corophium volutator* showed a large decrease in MO_2 from an acute reac-

tion to the change in salinity, to an apparent coping strategy (either by acclimating or reducing other processes). The comparison in size classes also showed an apparent better ability for larger individuals to cope with both short and medium term salinity changes better than smaller *C. volutator*. These same patterns were not seen with *G. marinus*. The length of exposure to the change in salinity did not reveal significant differences, however, the size classes were more telling in the 5 day experiments than the 4 hour tests. A lower MO_2 was evident with larger individuals in the medium term experiments. This might indicate different mechanisms at work for larger *G. marinus*. As for *G. chevreuxi*, the difference between the MO_2 of smaller versus larger individuals was evident for both short and medium term experiments. There did not appear to be any obvious acclimatory ability from the short to medium term results for *G. chevreuxi*.

During preliminary trials, the mortality at extreme salinities (e.g. *G. marinus* at 2 ppt) was higher than expected. An increase in survivorship was noted when the animals were feed during the medium term experiments. The effect of food deprivation has been shown to affect the results of oxygen consumption in cuttlefish, *Sepia officinalis* (Linnaeus) (Grigoriou & Richardson 2009). Even though it is common practice to withhold food during experiment trials, the results of the current experiments show how increased length of exposure to stressful conditions can be mediated by access to food. Stress can be a driver for evolution, and adaptation under these conditions may compensate for the induced stress (Feder 2007). The results of this chapter add to this view, that species exposed to environmental change which can induce a stress response in the form of increased respiratory rates, can lower the metabolic costs when the salinity stress is prolonged by acclimatizing to these altered conditions.

Thermal limits

The aim of Chapter 3 was to measure the effect of biotic and abiotic factors upon physiological tolerances across the three amphipod species. It was expected that *G. chevreuxi* would have the narrowest thermal range, with *C. volutator* exhibiting the widest thermal range due to its estuarine mud flat habitat, and *G. marinus* was expected to have a thermal range between that of the other two amphipod species. Thus, are the critical thermal limits modified by the interaction between salinity and temperature (both acclimation and seasonal)? The thermal limits were modified by a number of factors, differing, like the previous chapter, upon species. *Corophium volutator* showed the greatest tolerance to high temperatures, individuals surviving above 43°C before death occurred. Season and acclimation (to test temperatures) were seen to modulate the relationship between salinity and thermal maximum and minimum for *C. volutator*. Unexpectedly, *G. marinus* was found to have the largest thermal range, mainly due to its high tolerance of low temperatures. Season was seen to be an important factor for both CTMax and CTMin for *G. marinus*. Acclimatory ability of *G. chevreuxi* significantly affected this species thermal tolerance. Salinity was the most significant factor affecting the CTMin of *G. chevreuxi*. Differences between the sexes were not noted for either *C. volutator* or for *G. marinus*, however, significant differences were found for *G. chevreuxi*. Males were found to have a significantly lower endpoint of 'no movement' for minimal temperatures.

The received view (see del Rio 2008) of oxygen limitation of thermal tolerance taken by several studies (e.g. Pörtner 2002, Pörtner et al. 2005, Pörtner & Knust 2007) has been greatly debated (see Klok & Chown 2003, Chown & Terblanche 2006). This is because Pörtner (2002) states that the thermal limits of animals are set by the transition

to anaerobic metabolism. A comparative experiment between a beetle, *Gonocephalum simplex* (Fabricius) and a terrestrial isopod, *Armadillidium vulgare* (Latreille), set out to test this view (Klok & Chown 2003). The results of these experiments showed that the CTMax of *A. vulgare* was limited by its passive oxygen delivery system (pleopod beating), similar to the amphipods in the current thesis. However, *G. simplex* alters its respiration via opening and closing of spiracles and the CTMax did not noticeably decline, even at the lowest oxygen concentrations (Klok & Chown 2003). This study does not support the absolute generality of oxygen limited thermal tolerance for insects, however, it does support this view for crustaceans. Therefore, it is important to view the physiological experiments in this thesis as interrelated. For example, the MO_2 of *G. chevreuxi* increased after 5 days of exposure to 15 ppt, compared to 4 hours of exposure. The thermal limits of *G. chevreuxi* were also seen to be significantly lower at 20 ppt compared to the other two test salinities. Therefore, it is possible that the increased MO_2 was related to stress caused by low oxygen availability, further limiting the thermal range of *G. chevreuxi*.

Abundance-occupancy relationship

The aim of Chapter 4 was to quantify the spatial and temporal variability in the abundance and distribution of the three amphipod species. Multiple questions were posed: does the abundance and occupancy of these amphipods change over time? Do all sites sampled show the same population density? Is the composition of these populations similar across all sites and sampling dates?

The results of this chapter revealed that density did change temporally and spatially. Gaston & Curnutt (1998) found that within a large dataset for birds across the U.K., a positive abundance-size range was possible, without having a minimum density at all

sites. Meaning, that some sites can show a lower abundance, but overall the widespread species will show a positive relationship due to higher maximum abundances. This relationship can also be seen in the current work. Sites which were found to have the highest overall abundances did not do so across individual sampling dates.

Further to this, personal observations of *C. volutator* reinforces the variability noted previously. *Corophium volutator* was not sampled from the Plym estuary due to the complete absence over the summer of 2006, however, subsequent *Gammarus* sampling showed the reappearance of *C. volutator* in small numbers. Research by Fox et al. (2009) showed a reduction in *Corophium* spp. abundance in a eutrophic sub-estuary in Waquoit Bay, Massachusetts. The absence of *C. volutator* from ideal habitat and subsequent recolonisation, might indicate a pollution event in the Plym estuary which severely affected the population.

All sites, for all species, were dominated by females. *C. volutator* was seen to have between one and four generations per year (e.g. Segerstråle 1959, McLusky 1968, Fish & Mills 1979) and the data collected presently confirms that this species was likely to have two generations per year. *G. marinus* is considered to have continuous reproduction, with up to 4 broods per year (Marques et al. 2003, Maranhao et al. 2001, Maranhao & Marques 2003, Marques & Nogueira 1991, van Maren 1975). In the current study, there was evidence that *G. marinus* reproduces throughout the year in the studied sites. The research available for *G. chevreuxi* is limited with respect to mating behaviour and sexual dimorphism, however, the information available shows a biased sex ratio towards females, with only 22% of the population being male, with breeding occurring throughout the year (Subida et al. 2005). However, *G. chevreuxi* was seen to have a single peak in juveniles during the month of June for both sampling years. What

is evident from previous and current research, is that there is a tremendous amount of plasticity in the timing and number of reproductive events for all three amphipod species.

Therefore, the variability within these amphipod populations is linked spatially, temporally and demographically. These are important factors to consider for the management of these key ecological species, as they represent important positions in the food web as grazers and predator (e.g. Dick et al. 2005, Kelly et al. 2002), as well as prey for birds (Peer et al. 1986) and for commercially important fish (e.g. Hampel et al. 2009).

Conceptual model

The central question remains: can these physiological limits be used to predict the presence or absence of amphipods given habitat information? This was not conclusively addressed, as the BLR showed that the dataset was not large enough to elucidate the factors driving the current presence/absence data. Therefore, given the data available, the question can be amended to: can these physiological limits be used to predict the presence or absence of amphipods given population demographics and habitat information? The answer to this question is yes, given enough data. There is a large temporal component to the abundance and distribution of these amphipods. The two years of data were not enough to smooth out the different findings from June 2006 to June 2007 compared to August 2007 to June 2008.

Consideration of scale

Viewing assemblages from a large-scale perspective is often referred to as ‘macro’. Macroecology is an older discipline than macrophysiology, at least partly due to the availability of large datasets such as that for British birds being available before large

scale physiological studies. With increased impetus on climate change and the need to understand how ecosystem functioning will be altered by these changes, macrophysiology is an important field (Gaston et al. 2009). In this thesis, the use of ‘macro’ is avoided intentionally. Although the use of three different amphipod species from contrasting estuaries may seem somewhat macrophysiological, a larger scale across the U.K. is needed. The lack of robust findings in the model chapter makes the need for a larger dataset clear. A longer sampling regime, or a more direct sampling regime across a large geographic area such as the *in situ* physiological measurements obtained for porcelain crabs by Stillman & Somero (2000), may have provided more definitive results when it came time to model the data.

Scale is a critical factor when attempting to discern patterns in nature. The origins of metabolic theory are many and have evolved considerably to what is used today in ecology (Hemmingsen 1950, 1960, Lochhead 1977, Wu 1977, Schmidt-Nielsen 1977, Wilkie 1977, Prange 1977). Brown et al. (2004) proposed a metabolic theory of ecology, based on these and other earlier attempts. This theory contends that all animals metabolic rates are restricted by how they acquire, transform and expend energy, with a scaling exponent of $\frac{3}{4}$. The universality of this theory is greatly debated (e.g. O’Connor et al. 2007, Clarke 2006, van der Meer 2006). O’Connor et al. (2007) contends that although Brown et al. (2004) justify the theory on first principles, fundamental assumptions are still being made. For example, O’Connor et al. (2007) disagree with the assumption that the minimization of costs are linked mechanistically to the allometric hypothesis. Furthermore, del Rio (2008) contends that the basic argument lies in how researchers view biological system functioning. For some researchers, all animals functioning are constrained by the laws of energy acquisition and expenditure. Others

believe that the root of physiological limits can be based on several different mechanisms and treat each case separately, grouping similar cases together without making overall biological generalities (del Rio 2008).

The intention in the current research, of assessing not only adult physiology, but juvenile as well, was to view the small scale intra and interspecific variation. Having accomplished this, the work can form the basis for much larger, long term datasets, which can be used to advance macroecological and macrophysiological disciplines and enhance the debate over metabolic scaling as a whole.

Appendix A

Appendix A

Table A.1: Results of the GLM for CTMax endpoint of 'stop swimming' for all three species, the results for Gammarids as well as the the endpoint of 'death'

Factor	'stop swimming'				Gammarids				'death'			
	All species								All species			
	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P
Mass	1	7.731	4.55	0.034	1	6.417	4.39	0.037	1	6.718	3.51	0.062
Species	2	13.635	8.03	<0.001	1	14.732	10.09	0.002	2	210.321	109.80	<0.001
Season	1	64.201	37.79	<0.001	1	68.211	46.71	<0.001	1	3.518	1.84	0.176
Salinity	2	19.175	11.29	<0.001	2	5.658	3.87	0.022	2	1.320	0.69	0.503
Temperature	1	598.515	352.26	<0.001	1	372.579	255.13	<0.001	1	153.615	80.19	<0.001
Species*season	2	20.436	12.03	<0.001	1	32.002	21.91	<0.001	2	5.943	3.10	0.046
Species*salinity	4	20.718	12.19	<0.001	2	34.817	23.84	<0.001	4	7.577	3.96	0.004
Species*temp	2	29.502	17.36	<0.001	1	57.879	39.63	<0.001	2	14.859	7.76	0.001
Season * Salinity	2	6.999	4.12	0.017	2	5.875	4.02	0.019	2	3.796	1.98	0.139
Season* temp	1	0.000	0.00	0.987	1	0.016	0.01	0.918	1	10.209	5.33	0.022
Salinity * temp	2	8.041	4.73	0.009	2	5.714	3.91	0.021	2	14.735	7.69	0.001
Season*salinity*temp	2	6.394	3.76	0.024	2	12.764	8.74	<0.001	2	7.056	3.68	0.026
Species*season*salinity	4	7.982	4.70	0.001	2	11.369	7.79	0.001	4	5.689	2.97	0.020
Species*season*temp	2	42.944	25.27	<0.001	1	85.915	58.83	<0.001	2	22.968	11.99	<0.001
Species*salinity*temp	4	12.244	7.21	<0.001	2	10.709	7.33	0.001	4	7.749	4.05	0.003
Species*season*salinity*temp	4	8.681	5.11	0.001	2	3.163	2.17	0.117	4	6.328	3.30	0.011
Error	365				239				365			

A.1. CRITICAL THERMAL MAXIMUM

Table A.2: Results of the ANOVA GLM for *C. volutator* CTMax for the endpoint of ‘stop swimming’

Factor	DF	MS	F	P
Mass	1	13.908	6.72	0.011
Season	1	9.999	4.83	0.030
Salinity	2	23.172	11.20	<0.001
Temperature	1	236.002	114.10	<0.001
Season * Salinity	2	6.841	3.31	0.040
Season* temp	1	0.000	0.00	0.999
Salinity * temp	2	17.420	8.42	<0.001
Season*salinity*temp	2	7.562	3.66	0.029
Error	125			

Table A.3: Results of the GLM for *C. volutator* CTMax for the endpoint of ‘stop swimming’ including life stage

Factor	DF	MS	F	P
Mass	1	1.235	0.45	0.505
Temperature	1	169.122	61.09	<0.001
Life stage	2	2.316	0.84	0.435
Temp*life stage	2	0.910	0.33	0.720
Error	131			

A.1. CRITICAL THERMAL MAXIMUM

Table A.4: Results of the ANOVA GLM for *G. marinus* CTMax for the endpoint of 'stop swimming'

Factor	DF	MS	F	P
Mass	1	5.050	3.88	0.051
Season	1	93.563	71.95	<0.001
Salinity	2	23.681	18.21	<0.001
Temperature	1	66.919	51.46	<0.001
Season * Salinity	2	0.485	0.37	0.690
Season* temp	1	40.832	31.40	<0.001
Salinity * temp	2	12.821	9.86	<0.001
Season*salinity*temp	2	4.134	3.18	0.045
Error	114			

Table A.5: Results of the ANOVA GLM for *G. marinus* CTMax for the endpoint of 'stop swimming' including life stage

Factor	DF	MS	F	P
Mass	1	7.221	7.67	0.007
Season	1	105.670	112.17	<0.001
Salinity	2	19.871	21.09	<0.001
Temperature	1	50.196	53.28	<0.001
Life stage	1	1.544	1.64	0.203
Season * Salinity	2	0.101	0.11	0.898
Season* temp	1	32.816	34.84	<0.001
Season*life stage	1	2.401	2.55	0.114
Salinity * temp	2	15.670	16.63	<0.001
Salinity*life stage	2	0.715	0.76	0.471
Temp*life stage	1	1.328	1.41	0.238
Season*salinity*temp	2	1.667	1.77	0.176
Season*salinity*life stage	2	2.121	2.25	0.111
Season*temp*life stage	1	0.848	0.90	0.345
Salinity*temp*life stage	2	2.579	2.74	0.070
Season*salinity*temp*life stage	2	0.415	0.44	0.645
Error	99			

A.1. CRITICAL THERMAL MAXIMUM

Table A.6: Results of the ANOVA GLM for *G. chevreuxi* CTMax for the endpoint of 'stop swimming'

Factor	DF	MS	F	P
Mass	1	16.820	11.25	0.001
Season	1	5.771	3.86	0.052
Salinity	2	13.534	9.06	<0.001
Temperature	1	316.129	211.52	<0.001
Season * Salinity	2	15.747	10.54	<0.001
Season* temp	1	35.195	23.55	<0.001
Salinity * temp	2	3.386	2.27	0.108
Season*salinity*temp	2	10.602	7.09	0.001
Error	124			

Table A.7: Results of the GLM for *G. chevreuxi* CTMax for the endpoint of 'stop swimming' including life stage

Factor	DF	MS	F	P
Mass	1	8.893	5.86	0.017
Season	1	3.628	2.39	0.125
Salinity	2	10.989	7.24	0.001
Temperature	1	250.723	165.24	<0.001
Life stage	1	0.090	0.06	0.808
Season * Salinity	2	12.888	8.49	<0.001
Season* temp	1	30.033	19.79	<0.001
Season*life stage	1	3.682	2.43	0.122
Salinity * temp	2	2.334	1.54	0.219
Salinity*life stage	2	2.436	1.61	0.205
Temp*life stage	1	0.150	0.10	0.754
Season*salinity*temp	2	7.387	4.87	0.009
Season*salinity*life stage	2	2.598	1.71	0.185
Season*temp*life stage	1	1.740	1.15	0.287
Salinity*temp*life stage	2	0.019	0.01	0.987
Season*salinity*temp*life stage	2	0.037	0.02	0.976
Error	110			

A.2 Critical thermal minimum

Table A.8: Results of the ANOVA GLM for CTMin endpoint of 'no movement' for all three species, the results for Gammarids as well as the the endpoint of 'death'

Factor	'stop swimming'				Gammarids				'death'			
	All species				All species				All species			
	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P
Mass	1	263.15	61.58	<0.001	1	246.26	57.04	<0.001	1	160.48	26.12	<0.001
Species	2	1138.72	266.45	<0.001	1	362.06	83.86	<0.001	2	1143.71	186.12	<0.001
Season	1	77.05	18.03	<0.001	1	172.20	39.39	<0.001	1	19.95	3.25	0.072
Salinity	2	53.65	12.55	<0.001	2	6.66	1.54	0.216	2	79.78	12.98	<0.001
Temperature	1	105.69	24.73	<0.001	1	24.07	5.57	0.019	1	45.75	7.46	0.007
Species*season	2	99.23	23.22	<0.001	1	90.52	20.97	0.003	2	124.47	20.26	<0.001
Species*salinity	4	31.76	7.43	<0.001	2	25.43	5.89	0.001	4	41.11	6.69	<0.001
Species*temp	2	18.19	4.26	0.015	1	0.12	0.03	0.868	2	73.81	12.01	<0.001
Season * Salinity	2	43.93	10.28	<0.001	2	25.79	5.97	0.003	2	40.24	6.55	0.002
Season * temp	1	49.02	11.47	0.001	1	3.40	0.79	0.376	1	6.41	1.04	0.308
Salinity * temp	2	119.38	27.93	<0.001	2	69.73	16.15	<0.001	2	63.82	10.39	<0.001
Season*salinity*temp	2	7.90	1.85	0.159	2	13.41	3.11	0.047	2	2.95	0.48	0.619
Species*season*salinity	4	57.76	13.51	<0.001	2	39.03	9.04	<0.001	4	29.82	4.85	0.001
Species*season*temp	2	24.32	5.69	0.004	1	1.23	0.28	0.595	2	10.96	1.78	0.170
Species*salinity*temp	4	14.61	3.42	0.009	2	23.95	5.55	0.004	4	5.80	0.94	0.439
Species*season*salinity*temp	4	68.51	16.03	<0.001	2	62.87	14.56	<0.001	4	49.93	8.13	<0.001
Error	346				223				346			

A.2. CRITICAL THERMAL MINIMUM

Table A.9: Results of the ANOVA GLM for *C. volutator* CTMin for the endpoint of 'no movement'

Factor	DF	MS	F	P
Mass	1	128.014	39.95	<0.001
Season	1	11.677	3.64	0.059
Salinity	2	118.030	36.84	<0.001
Temperature	1	123.337	38.49	<0.001
Season * Salinity	2	130.093	40.60	<0.001
Season* temp	1	109.215	34.09	<0.001
Salinity * temp	2	52.540	16.40	<0.001
Season*salinity*temp	2	62.754	19.59	<0.001
Error	121			

Table A.10: Results of the ANOVA GLM for *C. volutator* CTMin for the endpoint of 'no movement' including life stage

Factor	DF	MS	F	P
Mass	1	17.230	2.67	0.105
Salinity	2	30.937	4.80	0.010
Temperature	1	117.281	18.18	<0.001
Life stage	2	11.719	1.82	0.167
Salinity * temp	2	43.490	6.74	0.002
Salinity*life stage	4	10.543	1.63	0.170
Temp*life stage	2	6.029	0.93	0.396
Salinity*temp*life stage	4	11.796	1.83	0.128
Error	115			

A.2. CRITICAL THERMAL MINIMUM

Table A.11: Results of the ANOVA GLM for *G. marinus* CTMin for the endpoint of 'no movement'

Factor	DF	MS	F	P
Mass	1	225.485	53.60	<0.001
Season	1	237.844	56.54	<0.001
Salinity	2	3.102	0.74	0.481
Temperature	1	12.704	3.02	0.085
Season * Salinity	2	2.514	0.60	0.552
Season* temp	1	4.099	0.97	0.326
Salinity * temp	2	25.736	6.12	0.003
Season*salinity*temp	2	9.148	2.17	0.119
Error	102			

Table A.12: Results of the ANOVA GLM for *G. marinus* CTMin for the endpoint of 'no movement' including life stage

Factor	DF	MS	F	P
Mass	1	124.120	30.79	<0.001
Season	1	207.679	51.52	<0.001
Salinity	2	5.407	1.34	0.267
Temperature	1	11.782	2.92	0.091
Life stage	1	4.222	1.05	0.309
Season * Salinity	2	4.319	1.07	0.347
Season* temp	1	0.581	0.14	0.705
Season*life stage	1	14.571	3.61	0.061
Salinity * temp	2	24.338	6.04	0.003
Salinity*life stage	2	5.145	1.28	0.284
Temp*life stage	1	0.294	0.07	0.788
Season*salinity*temp	2	13.297	3.30	0.041
Season*salinity*life stage	2	0.742	0.18	0.832
Season*temp*life stage	1	13.809	3.43	0.068
Salinity*temp*life stage	2	5.046	1.25	0.291
Season*salinity*temp*life stage	2	6.534	1.62	0.203
Error	89			

A.2. CRITICAL THERMAL MINIMUM

Table A.13: Results of the ANOVA GLM for *G. chevreuxi* CTMin for the endpoint of 'no movement'

Factor	DF	MS	F	P
Mass	1	21.819	4.92	0.028
Season	1	6.421	1.45	0.231
Salinity	2	30.468	6.86	0.002
Temperature	1	10.179	2.29	0.133
Season * Salinity	2	70.525	15.89	<0.001
Season* temp	1	0.213	0.05	0.827
Salinity * temp	2	67.725	15.26	<0.001
Season*salinity*temp	2	75.543	17.02	<0.001
Error	120			

Table A.14: Results of the ANOVA GLM for *G. chevreuxi* CTMin for the endpoint of 'no movement' including life stages

Factor	DF	MS	F	P
Mass	1	7.952	2.15	0.146
Season	1	12.944	3.50	0.064
Salinity	2	15.173	4.10	0.019
Temperature	1	6.337	1.71	0.193
Sex	1	42.123	11.39	0.001
Season * Salinity	2	66.259	17.91	<0.001
Season* temp	1	0.923	0.25	0.618
Season*Sex	1	2.373	0.64	0.425
Salinity * temp	2	54.735	14.80	<0.001
Salinity*sex	2	14.367	3.88	0.024
Temp*sex	1	7.884	2.13	0.147
Season*salinity*temp	2	52.513	14.20	<0.001
Season*salinity*sex	2	1.883	0.51	0.603
Season*temp*sex	1	0.702	0.19	0.664
Salinity*temp*sex	2	17.633	4.77	0.010
Season*salinity*temp*sex	2	10.712	2.90	0.060
Error	107			

References

- Addo-Bediako, A., Chown, S. L. & Gaston, K. J. (2000), 'Thermal tolerance, climatic variability and latitude', *Proceedings of the Royal Society of London Series B - Biological Sciences* **267**, 739–745.
- Agnew, D. & Taylor, A. C. (1985), 'The effects of oxygen tension on the physiology and distribution of *Echinogammarus pirloti* (Sexton & Spooner) and *E. obtusatus* (Dahl) (Crustacea: Amphipoda)', *Journal of Experimental Marine Biology and Ecology* **87**, 169–190.
- Aikins, S. & Kikuchi, E. (2001), 'Studies on habitat selection by amphipods using artificial substrates within an estuarine environment', *Hydrobiologia* **457**, 77–86.
- Akesson, B. & Costlow, J. D. (1978), 'Effects of temperature and salinity on life-cycle of *Ophryotrocha diadema* (Polychaeta, Dorvilleidae)', *Ophelia* **17**, 215–229.
- Anastacio, P. M., Goncalves, S. C., Pardal, M. A. & Marques, J. C. (2003), 'A model for amphipod (*Talitrus saltator*) population dynamics', *Estuarine Coastal and Shelf Science* **58**, 149–157.
- Andrewartha, H. (1970), *Introduction to the Study of Animal Populations*, Second edn, Chapman and Hall Ltd, London.
- Andrewartha, H. & Birch, L. (1954), *The distribution and abundance of animals*, First edn, The University of Chicago Press, Chicago.
- Andrewartha, H. & Birch, L. (1984), *The ecological web*, First edn, The University of Chicago Press, Chicago and London.
- Andrews, R. M. (1998), 'Geographic variation in field body temperature of sceloporine lizards', *Journal of Thermal Biology* **23**, 329–334.
- Attrill, M. J. (2002), 'A testable linear model for diversity trends in estuaries', *Journal of Animal Ecology* **71**, 262–269.

- Attrill, M. J. & Power, M. (2004), 'Partitioning of temperature resources amongst an estuarine fish assemblage', *Estuarine Coastal and Shelf Science* **61**, 725–738.
- Attrill, M. J. & Rundle, S. D. (2002), 'Ecotone or ecocline: Ecological boundaries in estuaries', *Estuarine Coastal and Shelf Science* **55**, 929–936.
- Attrill, M. J., Stafford, R. & Rowden, A. A. (2001), 'Latitudinal diversity patterns in estuarine tidal flats: Indications of a global cline', *Ecography* **24**, 318–324.
- Bailey, N. (1995), *Statistical methods in biology*, Cambridge University Press, Cambridge.
- Bakker, V. J. & Kelt, D. A. (2000), 'Scale-dependent patterns in body size distributions of neotropical mammals', *Ecology* **81**, 3530–3547.
- Bamber, R. N. (1985), 'The autoecology of *Cyathura carinata* (Crustacea, Isopoda) in a cooling water discharge lagoon', *Journal of the Marine Biological Association of the United Kingdom* **65**, 181–194.
- Bard, E.-S., Tappin, A. & Achterberg, E. (2008), 'Distribution and seasonal variability of dissolved organic nitrogen in two estuaries in SW England', *Marine Chemistry* **110**, 153–164.
- Barnes, R. (1974), *Estuarine biology*, First edn, Edward Arnold, London.
- Barnes, R., Williams, A., Little, C. & Dorey, A. (1979), An ecological study of Swanpool, Falmouth. Population fluctuations of some dominant macrofauna, in R. Jefferies & A. Davy, eds, 'Ecological Processes in the Coastal Environment', Blackwell, London, pp. 177–197.
- Bell, R. G., Hume, T. M., Dolphin, T. J., Green, M. O. & Walters, R. A. (1997), 'Characterisation of physical environmental factors on an intertidal sandflat, Manukau Harbour, New Zealand', *Journal of Experimental Marine Biology and Ecology* **216**, 11–31.
- Bellan-Santini, D. (2009), '*Gammarus marinus* Leach, 1815'. Accessed through: World Register of Marine Species <http://www.marinespecies.org/aphia.php?p=taxdetails&id=102283>.

- Bergstrom, U., Englund, G. & Bonsdorff, E. (2002), 'Small-scale spatial structure of Baltic Sea zoobenthos - Inferring processes from patterns', *Journal of Experimental Marine Biology and Ecology* **281**, 123–136.
- Bernardo, J. & Spotila, J. R. (2006), 'Physiological constraints on organismal response to global warming: mechanistic insights from clinally varying populations and implications for assessing endangerment', *Biology Letters* **2**, 135–139.
- Bettison, J. & Davenport, J. (1976), 'Salinity preference in gammarid amphipods with special reference to *Marinogammarus marinus* (Leach)', *Journal of the Marine Biological Association of the United Kingdom* **56**, 135–142.
- Bilton, D. T., Paula, J. & Bishop, J. D. D. (2002), 'Dispersal, genetic differentiation and speciation in estuarine organisms', *Estuarine Coastal and Shelf Science* **55**, 937–952.
- Blackburn, T. M. (2004), 'Method in macroecology', *Basic and Applied Ecology* **5**, 401–412.
- Blackburn, T. M., Cassey, P. & Gaston, K. J. (2006), 'Variations on a theme: Sources of heterogeneity in the form of the interspecific relationship between abundance and distribution', *Journal of Animal Ecology* **75**, 1426–1439.
- Blackburn, T. M. & Gaston, K. J. (1998), 'Some methodological issues in macroecology', *American Naturalist* **151**, 68–83.
- Bolnick, D. I., Yang, L. H., Fordyce, J. A., Davis, J. M. & Svanback, R. (2002), 'Measuring individual-level resource specialization', *Ecology* **83**, 2936–2941.
- Braby, C. E. & Somero, G. N. (2006), 'Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *mytilus*)', *Journal of Experimental Biology* **209**(1), 2554–2566.
- Brenko, M. H. & Calabrese, A. (1969), 'The combined effect of salinity and temperature on larvae of the mussel *Mytilus edulis*', *Marine Biology* **4**, 224–226.

- Brooks, S. J. & Mills, C. L. (2006), 'Gill Na⁺, K⁺-ATPase in a series of hyper-regulating gammarid amphipods. Enzyme characterisation and the effects of salinity acclimation', *Comparative Biochemistry and Physiology A - Molecular & Integrative Physiology* **144**, 24–32.
- Brown, J. H. (1984), 'On the relationship between abundance and distribution of species', *American Naturalist* **124**, 255–279.
- Brown, J. H. (1995), *Macroecology*, The University of Chicago Press, Chicago.
- Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. & West, G. B. (2004), 'Toward a metabolic theory of ecology', *Ecology* **85**, 1771–1789.
- Brown, J. H., Gillooly, J., West, G. & Savage, V. (2003), The next step in macroecology: From general empirical patterns to universal ecological laws, in T. M. Blackburn & K. J. Gaston, eds, 'Macroecology: Concepts and consequences', First edn, Blackwell, Oxford.
- Brown, J. H. & Maurer, B. A. (1989), 'Macroecology - The division of food and space among species on continents', *Science* **243**, 1145–1150.
- Brujls, M. C. M., Kelleher, B., van der Velde, G. & de Vaate, A. B. (2001), 'Oxygen consumption, temperature and salinity tolerance of the invasive amphipod *Dikerogammarus villosus*: Indicators of further dispersal via ballast water transport', *Archiv fur Hydrobiologie* **152**, 633–646.
- Buchanan, J. A., Stewart, B. A. & Davies, B. R. (1988), 'Thermal acclimation and tolerance to lethal high temperature in the mountain stream amphipod *Paramelita nigroculus* (Barnard)', *Comparative Biochemistry and Physiology A - Molecular & Integrative Physiology* **89**, 425–431.
- Buck, A. (1997), *An inventory of UK estuaries. Volume 6. Southern England*, Joint Conservation Committee, Peterborough.
- Calosi, P., Bilton, D. T., Spicer, J. I. & Atfield, A. (2008), 'Thermal tolerance and geographical range size in the *Agabus brunneus* group of European diving beetles (Coleoptera : Dytiscidae)', *Journal of Biogeography* **35**, 295–305.

- Campbell, J. I. & Meadows, P. S. (1974), 'Gregarious behavior in a benthic marine amphipod (*Corophium volutator*)', *Experientia* **30**, 1396–1397.
- Castaneda, L. E., Lardies, M. A. & Bozinovic, F. (2004), 'Adaptive latitudinal shifts in the thermal physiology of a terrestrial isopod', *Evolutionary Ecology Research* **6**, 579–593.
- Chown, S. L. & Gaston, K. J. (1999), 'Exploring links between physiology and ecology at macro-scales: The role of respiratory metabolism in insects', *Biological Reviews* **74**, 87–120.
- Chown, S. L., Gaston, K. J. & Robinson, D. (2004), 'Macrophysiology: Large-scale patterns in physiological traits and their ecological implications', *Functional Ecology* **18**, 159–167.
- Chown, S. L., Marais, E., Terblanche, J. S., Klok, C. J., Lighton, J. R. B. & Blackburn, T. M. (2007), 'Scaling of insect metabolic rate is inconsistent with the nutrient supply network model', *Functional Ecology* **21**, 282–290.
- Chown, S. L. & Terblanche, J. S. (2006), 'Physiological diversity in insects: Ecological and evolutionary contexts', *Advances in Insect Physiology* **33**, 50–152.
- Clarke, A. (1991), 'What is cold adaptation and how should we measure it?', *American Zoologist* **31**, 81–92.
- Clarke, A. (2006), 'Temperature and the metabolic theory of ecology', *Functional Ecology* **20**, 405–412.
- Clarke, K. R. & Ainsworth, M. (1993), 'A method of linking multivariate community structure to environmental variables', *Marine Ecology Progress Series* **92**, 205–219.
- Clason, B., Langston, W. J. & Zauke, G. P. (2004), 'Bioaccumulation of trace metals in the amphipod *Chaetogammarus marinus* (Leach, 1815) from the Avon and Tamar estuaries (UK): Comparison of two-compartment and hyperbolic toxicokinetic models', *Marine Environmental Research* **57**, 171–195.

- Cofre, H. L., Bohning-Gaese, K. & Marquet, P. A. (2007), 'Rarity in chilean forest birds: Which ecological and life-history traits matter?', *Diversity and Distributions* **13**, 203–212.
- Compton, T. J., Rijkenberg, M. J. A., Drent, J. & Piersma, T. (2007), 'Thermal tolerance ranges and climate variability: A comparison between bivalves from differing climates', *Journal of Experimental Marine Biology and Ecology* **352**, 200–211.
- Conlan, K. E. (1991), 'Precopulatory mating-behaviour and sexual dimorphism in the amphipod Crustacea', *Hydrobiologia* **223**, 255–282.
- Cowles, R. & Bogert, C. (1944), 'A preliminary study of the thermal requirements of desert reptiles', *Bulletin of the American Museum of Natural History* **83**, 261–296.
- Cowling, J. E., Spicer, J. I., Weeks, J. M. & Gaston, K. J. (2003), 'Environmental tolerances of an invasive terrestrial amphipod, *Arcitalitrus dorrieni* (Hunt) in Britain', *Comparative Biochemistry and Physiology A - Molecular & Integrative Physiology* **136**, 735–747.
- Cowling, J. E., Weeks, J. A., Spicer, J. I. & Gaston, K. J. (2004), 'Physiological tolerances of the euterrestrial amphipod *Arcitalitrus dorrieni* (Hunt) as a key to its geographical distribution? A test using mesocosms', *Comparative Biochemistry and Physiology A - Molecular & Integrative Physiology* **139**, 251–259.
- Crawford, G. I. (1937), 'The fauna of certain estuaries in West England and South Wales, with special reference to the Tanaidacea, Isopoda and Amphipoda', *Journal of the Marine Biological Association of the United Kingdom* **21**, 647–662.
- Crewe, T. L., Hamilton, D. J. & Diamond, A. W. (2001), 'Effects of mesh size on sieved samples of *Corophium volutator*', *Estuarine Coastal and Shelf Science* **53**, 151–154.
- Cunha, M. R. & Moreira, M. H. (1995), 'Macrobenthos of Potamogeton and Myriophyllum beds in the upper reaches of canal de Mira (Ria de Aveiro, NW Portugal): Community structure and environmental factors', *Netherlands Journal of Sea Research* **29**, 377–390.

- Darwin, C. (1859), *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*, Rh Value Publishing, London.
- Dedecker, A. P., Van Melckebeke, K., Goethals, P. L. M. & De Pauw, N. (2007), 'Development of migration models for macroinvertebrates in the Zwalm river basin (Flanders, Belgium) as tools for restoration management', *Ecological Modelling* **203**, 72–86.
- del Rio, C. M. (2008), 'Metabolic theory or metabolic models?', *Trends in Ecology & Evolution* **23**, 256–260.
- Dick, J. T. A., Johnson, M. P., McCambridge, S., Johnson, J., Carson, V. E. E., Kelly, D. W. & MacNeil, C. (2005), 'Predatory nature of the littoral amphipod *Echinogammarus marinus*: Gut content analysis and effects of alternative food and substrate heterogeneity', *Marine Ecology-Progress Series* **291**, 151–158.
- Dieleman, J. (1978), Swimming rhythms, migration and breeding cycles in the estuarine amphipods *Gammarus chevreuxi* and *Gammarus zaddachi*, in E. Naylor & R. G. Hartnoll, eds, 'Cyclic phenomena in marine plants and animals', Pergamon Press, Oxford, pp. 415–422.
- Diniz-Filho, J. A. F. & Torres, N. M. (2002), 'Phylogenetic comparative methods and the geographic range size - body size relationship in new world terrestrial carnivora', *Evolutionary Ecology* **16**, 351–367.
- Dorgelo, J. (1977), 'Comparative ecophysiology of two intertidal gammarids and the problem of zonation', *Crustaceana* **4**, 64–80.
- Elton, C. (1927), *Animal Ecology*, First edn, Sidwick & Jackson, London.
- Ernest, S. K. M., Enquist, B. J., Brown, J. H., Charnov, E. L., Gillooly, J. F., Savage, V., White, E. P., Smith, F. A., Hadly, E. A., Haskell, J. P., Lyons, S. K., Maurer, B. A., Niklas, K. J. & Tiffney, B. (2003), 'Thermodynamic and metabolic effects on the scaling of production and population energy use', *Ecology Letters* **6**, 990–995.

- Etilé, R. N., Kouassi, A. M., Aka, M. N., Pagano, M., N'Douba, V. & Kouassi, N. J. (2009), 'Spatio-temporal variations of the zooplankton abundance and composition in a West African tropical coastal lagoon (Grand-Lahou, Côte d'Ivoire)', *Hydrobiologia* **624**, 171–189.
- Falster, D. S., Murray, B. R. & Lepschi, B. J. (2001), 'Linking abundance, occupancy and spatial structure: An empirical test of a neutral model in an open-forest woody plant community in eastern Australia', *Journal of Biogeography* **28**, 317–323.
- Fangue, N. A., Hofmeister, M. & Schulte, P. M. (2006), 'Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*', *Journal of Experimental Biology* **209**, 2859–2872.
- Fangue, N. A., Richards, J. G. & Schulte, P. M. (2009), 'Do mitochondrial properties explain intraspecific variation in thermal tolerance?', *Journal of Experimental Biology* **212**, 514–522.
- Feder, M. E. (2007), 'Key issues in achieving an integrative perspective on stress', *Journal of Biosciences* **32**, 433–440.
- Fernandez, M. H. & Vrba, E. S. (2005), 'Macroevolutionary processes and biomic specialization: Testing the resource-use hypothesis', *Evolutionary Ecology* **19**, 199–219.
- Ferreira, S. M., Pardal, M. A., Lillebo, A. I., Cardoso, P. G. & Marques, J. C. (2004), 'Population dynamics of *Cyathura carinata* (Isopoda) in a eutrophic temperate estuary', *Estuarine Coastal and Shelf Science* **61**, 669–677.
- Fish, J. D. & Mills, A. (1979), 'Reproductive biology of *Corophium volutator* and *Corophium arenarium* (Crustacea, Amphipoda)', *Journal of the Marine Biological Association of the United Kingdom* **59**, 355–368.
- Flach, E. C. (1992), 'The influence of 4 macrozoobenthic species on the abundance of the amphipod *Corophium volutator* on tidal flats of the Wadden Sea', *Netherlands Journal of Sea Research* **29**, 379–394.

- Flach, E. C. & Debruin, W. (1993), 'Effects of *Arenicola marina* and *Cerastoderma edule* on distribution, abundance and population structure of *Corophium volutator* in Gullmarsfjorden Western Sweden', *Sarsia* **78**, 105–118.
- Foggo, A., Bilton, D. T. & Rundle, S. D. (2007), 'Do developmental mode and dispersal shape abundance-occupancy relationships in marine macroinvertebrates?', *Journal of Animal Ecology* **76**, 695–702.
- Foggo, A., Frost, M. T. & Attrill, M. J. (2003), 'Abundance-occupancy patterns in British estuarine macroinvertebrates', *Marine Ecology Progress Series* **265**, 297–302.
- Fontaine, C., Collin, C. L. & Dajoz, I. (2008), 'Generalist foraging of pollinators: Diet expansion at high density', *Journal of Ecology* **96**, 1002–1010.
- Forbes, M. R., Boates, J. S., McNeil, N. L. & Brison, A. E. (1996), 'Mate searching by males of the intertidal amphipod *Corophium volutator* (Pallas)', *Canadian Journal of Zoology* **74**, 1479–1484.
- Ford, A. T., Fernandes, T. F., Rider, S. A., Read, P. A., Robinson, C. D. & Davies, I. M. (2003), 'Reproduction in the amphipod, *Echinogammarus marinus*: A comparison between normal and intersex specimens', *Journal of the Marine Biological Association of the United Kingdom* **83**, 937–940.
- Fox, S. E., Teichberg, M., Olsen, Y. S., Heffner, L. & Valiela, I. (2009), 'Restructuring of benthic communities in eutrophic estuaries: Lower abundance of prey leads to trophic shifts from omnivory to grazing', *Marine Ecology Progress Series* **380**, 43–57.
- Freckleton, R. P., Gill, J. A., Noble, D. & Watkinson, A. R. (2005), 'Large-scale population dynamics, abundance-occupancy relationships and the scaling from local to regional population size', *Journal of Animal Ecology* **74**, 353–364.
- Frid, C. & James, R. (1989), 'The marine invertebrate fauna of the British coastal salt-marsh', *Holarctic Ecology* **12**, 9–15.

- Frisbie, M. P. & Lee, R. E. (1997), 'Inoculative freezing and the problem of winter survival for freshwater macroinvertebrates', *Journal of the North American Benthological Society* **16**, 635–650.
- Frost, M. T., Attrill, M. J., Rowden, A. A. & Foggo, A. (2004), 'Abundance-occupancy relationships in macrofauna on exposed sandy beaches: Patterns and mechanisms', *Ecography* **27**, 643–649.
- Gamble, J. C. (1970), 'Effect of low dissolved oxygen concentrations on ventilation rhythm of 3 tubicolous crustaceans, with special reference to phenomenon of intermittent ventilation', *Marine Biology* **6**, 121–127.
- Garland, T., Bennett, A. F. & Rezende, E. L. (2005), 'Phylogenetic approaches in comparative physiology', *Journal of Experimental Biology* **208**, 3015–3035.
- Gaston, K. J. (1996), 'The multiple forms of the interspecific abundance-distribution relationship', *Oikos* **76**, 211–220.
- Gaston, K. J. & Blackburn, T. M. (1999), 'A critique for macroecology', *Oikos* **84**, 353–368.
- Gaston, K. J. & Blackburn, T. M. (2000), *Patterns and processes in macroecology*, Blackwell Science, Oxford.
- Gaston, K. J., Blackburn, T. M., Greenwood, J. J. D., Gregory, R. D., Quinn, R. M. & Lawton, J. H. (2000), 'Abundance-occupancy relationships', *Journal of Applied Ecology* **37**, 39–59.
- Gaston, K. J., Blackburn, T. M. & Gregory, R. D. (1997b), 'Interspecific abundance-range size relationships: Range position and phylogeny', *Ecography* **20**, 390–399.
- Gaston, K. J., Blackburn, T. M., Gregory, R. D. & Greenwood, J. J. D. (1998), 'The anatomy of the interspecific abundance-range size relationship for the British avifauna: I. Spatial patterns', *Ecology Letters* **1**, 38–46.
- Gaston, K. J., Blackburn, T. M. & Lawton, J. H. (1997), 'Interspecific abundance range size relationships: An appraisal of mechanisms', *Journal of Animal Ecology* **66**, 579–601.

- Gaston, K. J., Blackburn, T. M. & Lawton, J. H. (1998), 'Aggregation and interspecific abundance-occupancy relationships', *Journal of Animal Ecology* **67**, 995–999.
- Gaston, K. J. & Chown, S. L. (1999), 'Why rapoport's rule does not generalise', *Oikos* **84**, 309–312.
- Gaston, K. J., Chown, S. L., Calosi, P., Bernardo, J., Bilton, D. T., Clarke, A., Clusella-Trullas, S., Ghalambor, C. K., Konarzewski, M., Peck, L. S., Porter, W. P., Pörtner, H. O., Rezende, E. L., Schulte, P. M., Spicer, J. I., Stillman, J. H., Terblanche, J. S. & van Kleunen, M. (2009), 'Macrophysiology: A conceptual reunification', *The American Naturalist* **174**, 595–612.
- Gaston, K. J., Chown, S. L. & Evans, K. L. (2008), 'Ecogeographical rules: Elements of a synthesis', *Journal of Biogeography* **35**, 483–500.
- Gaston, K. J. & Curnutt, J. L. (1998), 'The dynamics of abundance-range size relationships', *Oikos* **81**, 38–44.
- Gaston, K. J. & Spicer, J. I. (1998), 'Do upper thermal tolerances differ in geographically separated populations of the beachflea *Orchestia gammarellus* (Crustacea : Amphipoda)?', *Journal of Experimental Marine Biology and Ecology* **229**, 265–276.
- Gaston, K. J. & Spicer, J. I. (2001), 'The relationship between range size and niche breadth: A test using five species of *Gammarus* (Amphipoda)', *Global Ecology and Biogeography* **10**, 179–188.
- Gillooly, J. F., Brown, J. H., Allen, A. P., West, G. B., Charnov, E. L. & Savage, V. M. (2003), 'The central role of metabolism from genes to ecosystems', *Integrative and Comparative Biology* **43**, 923–923.
- Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. & Charnov, E. L. (2001), 'Effects of size and temperature on metabolic rate', *Science* **293**, 2248–2251.
- Gillooly, J. F., Charnov, E. L., West, G. B., Savage, V. M. & Brown, J. H. (2002), 'Effects of size and temperature on developmental time', *Nature* **417**, 70–73.

- Girisch, H. & Dennert, H. (1975), 'Simulation experiments on the migrations of *Gammarus zaddachi* and *G. chevreuxi*', *Bijdragen tot de dierkunde* **45**, 20–38.
- Girisch, H., Dieleman, J., Petersen, G. & Pinkster, S. (1974), 'The migration of two sympatric gammarid species in a French estuary', *Bijdragen tot de dierkunde* **44**, 239–273.
- Greenslade, P. J. M. (1983), 'Adversity selection and the habitat templet', *American Naturalist* **122**, 352–365.
- Gregory, R. D. & Gaston, K. J. (2000), 'Explanations of commonness and rarity in British breeding birds: Separating resource use and resource availability', *Oikos* **88**, 515–526.
- Grigoriou, P. & Richardson, C. A. (2009), 'Effect of body mass, temperature and food deprivation on oxygen consumption rate of common cuttlefish *Sepia officinalis*', *Marine Biology* **156**, 2473–2481.
- Haeckel, E. (1904), *The riddle of the universe at the close of the nineteenth century*, First edn, Watts & Co., London.
- Halcrow, K. & Boyd, C. (1967), 'The oxygen consumption and swimming activity of the amphipod *Gammarus oceanus* at different temperatures', *Comparative Biochemistry and Physiology A - Molecular & Integrative Physiology* **23**, 233–242.
- Hall, S., Raffaelli, D. & Thrush, S. F. (1994), Patchiness and distribution in shallow water benthic assemblages, in P. Giller, A. Hildrew & D. Raffaelli, eds, 'Aquatic ecology: Scale, pattern and process', Blackwell Science, pp. 333–375.
- Hampel, H., Elliott, M. & Cattrijsse, A. (2009), 'Macrofaunal communities in the habitats of intertidal marshes along the salinity gradient of the Schelde estuary', *Estuarine Coastal and Shelf Science* **84**, 45–53.
- Hanski, I. (1991), 'Reply to Nee, Gregory and May', *Oikos* **62**, 88–89.
- Hanski, I. (1998), 'Metapopulation dynamics', *Nature* **396**, 41–49.

- Hanski, I. & Gilpin, M. (1991), 'Metapopulation dynamics - Brief-history and conceptual domain', *Biological Journal of the Linnean Society* **42**, 3–16.
- Hanski, I. & Gyllenberg, M. (1997), 'Uniting two general patterns in the distribution of species', *Science* **275**, 397–400.
- Hanski, I., Kouki, J., Halkka, A., Ricklefs, R. E. & Schluter, D. (1993), Three explanations of the positive relationship between distribution and abundance of species, in 'Species diversity in ecological communities: Historical and geographical perspectives', First edn, University of Chicago Press, pp. 108–116.
- Harris, R. R. & Musko, I. B. (1999), 'Oxygen consumption, hypoxia, and tubedwelling in the invasive amphipod *Corophium curvispinum*', *Journal of Crustacean Biology* **19**, 224–234.
- Hartley, S. (1998), 'A positive relationship between local abundance and regional occupancy is almost inevitable (but not all positive relationships are the same)', *Journal of Animal Ecology* **67**, 992–994.
- Hemmingsen, A. (1950), 'The relation of standard (basal) energy metabolism to the total fresh weight of living organisms', *Report of the Steno Memorial Hospital and the Nordisk Insulinlaboratorium* **4**, 7–58.
- Hemmingsen, A. (1960), 'Energy metabolism as related to body size and respiratory surfaces, and its evolution', *Report of the Steno Memorial Hospital and the Nordisk Insulinlaboratorium* **9**, 7–110.
- Hewitt, J. E. & Thrush, S. F. (2009), 'Reconciling the influence of global climate phenomena on macrofaunal temporal dynamics at a variety of spatial scales', *Global Change Biology* **15**, 1911–1929.
- Hildrew, A. & Giller, P. (1994), Extensive surveys and patterns of persistence, in P. Giller, A. Hildrew & D. Raffaelli, eds, 'Aquatic ecology: Scale, pattern and process', Blackwell Science, Oxford, pp. 26–62.
- Hilton, C., Walde, S. J. & Leonard, M. L. (2002), 'Intense episodic predation by shorebirds may influence life history strategy of an intertidal amphipod', *Oikos* **99**, 368–376.

- Hoffmann, A. A. & Blows, M. W. (1994), 'Species borders: Ecological and evolutionary perspectives', *Trends in Ecology & Evolution* **9**, 223–227.
- Holt, A. R., Warren, P. H. & Gaston, K. J. (2004), 'The importance of habitat heterogeneity, biotic interactions and dispersal in abundance-occupancy relationships', *Journal of Animal Ecology* **73**, 841–851.
- Holt, R. D., Lawton, J. H., Gaston, K. J. & Blackburn, T. M. (1997), 'On the relationship between range size and local abundance: Back to basics', *Oikos* **78**, 183–190.
- Hou, Z., Fu, J. & Li, S. (2007), 'A molecular phylogeny of the genus *Gammarus* (Crustacea: Amphipoda) based on mitochondrial and nuclear gene sequences', *Molecular Phylogenetics and Evolution* **45**, 596–611.
- Huey, R. B. & Bennett, A. F. (1987), 'Phylogenetic studies of coadaptation - preferred temperatures versus optimal performance temperatures of lizards', *Evolution* **41**, 1098–1115.
- Hughes, R. G. & Gerdol, V. (1997), 'Factors affecting the distribution of the amphipod *Corophium volutator* in two estuaries in South-east England', *Estuarine Coastal and Shelf Science* **44**, 621–627.
- Hutchison, V. H. & Rowland, S. (1975), 'Thermal acclimation and tolerance in the mudpuppy, *Necturus maculosus*', *Journal of Herpetology* **9**, 367–368.
- Issartel, J., Voituron, Y., Odagescu, V., Baudot, A., Guillot, G., Ruaud, J. P., Renault, D., Vernon, P. & Hervant, F. (2006), 'Freezing or supercooling: How does an aquatic subterranean crustacean survive exposures at subzero temperatures?', *Journal of Experimental Biology* **209**, 3469–3475.
- Jenkins, S. R. (2005), 'Larval habitat selection, not larval supply, determines settlement patterns and adult distribution in two chthamalid barnacles', *Journal of Animal Ecology* **74**, 893–904.
- Jensen, K. T. & Andre, C. (1993), 'Field and laboratory experiments on interactions among an infaunal polychaete, *Nereis diversicolor*, and 2 amphipods, *Corophium*

- volutator* and *C. arenarium* - Effects on survival, recruitment and migration', *Journal of Experimental Marine Biology and Ecology* **168**, 259–278.
- Joy, M. K. & Death, R. G. (2004), 'Predictive modelling and spatial mapping of freshwater fish and decapod assemblages using GIS and neural networks', *Freshwater Biology* **49**, 1036–1052.
- Jørgensen, K. T., Sørensen, J. G. & Bundgaard, J. (2006), 'Heat tolerance and the effect of mild heat stress on reproductive characters in *Drosophila buzzatii* males', *Journal of Thermal Biology* **31**, 280–286.
- Kearney, M. & Porter, W. (2009), 'Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges', *Ecology Letters* **12**, 334–350.
- Kelly, D. W., Dick, J. T. A. & Montgomery, W. I. (2002), 'The functional role of *Gammarus* (Crustacea, Amphipoda): Shredders, predators, or both?', *Hydrobiologia* **485**, 199–203.
- Kinne, O. (1960), 'Growth, molting frequency, heart beat, number of eggs, and incubation time in *Gammarus zaddachi* exposed to different environments', *Crustaceana* **2**, 26–36.
- Kinne, O. (1970), *Marine Ecology*, Vol. 1, Wiley - Interscience, London.
- Klok, C. J. & Chown, S. L. (2003), 'Resistance to temperature extremes in sub-Antarctic weevils: Interspecific variation, population differentiation and acclimation', *Biological Journal of the Linnean Society* **78**, 401–414.
- Kockemann, B., Buschmann, H. & Leuschner, C. (2009), 'The relationships between abundance, range size and niche breadth in central European tree species', *Journal of Biogeography* **36**, 854–864.
- Kolar, C. S. & Rahel, F. J. (1993), 'Interaction of a biotic factor (predator presence) and an abiotic factor (low-oxygen) as an influence on benthic invertebrate communities', *Oecologia* **95**, 210–219.
- Korhonen, I. A. & Lagerspetz, K. Y. H. (1996), 'Heat shock response and thermal acclimation in *Asellus aquaticus*', *Journal of Thermal Biology* **21**, 49–56.

- Kotze, D. J., Niemela, J., O'Hara, R. B. & Turin, H. (2003), 'Testing abundance-range size relationships in European carabid beetles (Coleoptera, Carabidae)', *Ecography* **26**, 553–566.
- Krebs, C. (1978), *Ecology: The experimental analysis of distribution and abundance*, Second edn, Harper & Row Publishers, New York.
- Krebs, C. (1985), *Ecology: The experimental analysis of distribution and abundance*, Third edn, Harper & Row Publishers, New York.
- Krebs, C. (1994), *Ecology: The experimental analysis of distribution and abundance*, Fourth edn, Addison Wesley Longman Inc, Menlo Park California.
- Krog, J. (1954), 'The influence of seasonal environmental changes upon the metabolism, lethal temperature and rate of heart beat of *Gammarus limnaeus* (Smith) taken from an Alaskan lake', *Biological Bulletin of the Marine Biological Station (Woods Hole, Massachusetts)* **107**, 397–410.
- Lagerspetz, K. Y. H. & Bowler, K. (1993), 'Variation in heat tolerance in individual *Asellus aquaticus* during thermal acclimation', *Journal of Thermal Biology* **18**, 137–143.
- Lahdes, E. O. & Vainio, L. A. (2003), 'Effect of season on the acute thermal tolerance and thermal inactivation of membrane ($Mg^{2+}+Na^{+}+K^{+}$)- and Mg^{2+} -adenosine triphosphatase activity of the Baltic Sea amphipods, *Monoporeia affinis* and *Gammarus* spp', *Boreal Environment Research* **8**, 147–157.
- Landry, J., Bernier, D., Chretien, P., Nicole, L. M., Tanguay, R. M. & Marceau, N. (1982), 'Synthesis and degradation of heat shock proteins during development and decay of thermotolerance', *Cancer Research* **42**, 2457–2461.
- Lawrie, S. M., Raffaelli, D. G. & Emes, C. H. (2000), 'Small-scale patterns in the distribution of the amphipod *Corophium volutator* on the Ythan estuary, Aberdeenshire, Scotland', *Sarsia* **85**, 321–327.
- Lawton, J. H. (1991), 'From physiology to population-dynamics and communities', *Functional Ecology* **5**, 155–161.

- Layne, J. R., Claussen, D. L. & Manis, M. L. (1987), 'Effects of acclimation temperature, season, and time of day on the critical thermal maxima and minima of the crayfish *Orconectes rusticus*', *Journal of Thermal Biology* **12**, 183–187.
- Legendre, P. & Legendre, L. (1998), *Numerical ecology*, Second edn, Elsevier Science, Amsterdam.
- Lek, S. & Guegan, J. F. (1999), 'Artificial neural networks as a tool in ecological modelling, an introduction', *Ecological Modelling* **120**, 65–73.
- Levitan, D. R. (2000), 'Optimal egg size in marine invertebrates: Theory and phylogenetic analysis of the critical relationship between egg size and development time in echinoids', *American Naturalist* **156**, 175–192.
- Limia, J. & Raffaelli, D. (1997), 'The effects of burrowing by the amphipod *Corophium volutator* on the ecology of intertidal sediments', *Journal of the Marine Biological Association of the United Kingdom* **77**, 409–423.
- Lincoln, R. (1979), *British marine amphipoda: Gammaridea*, First edn, British Museum (Natural History), London.
- Lochhead, J. (1977), Unsolved problems of interest in the locomotion of Crustacea, in T. Pedley, ed., 'Scale effects in animal locomotion', Academic Press, London, pp. 257–268.
- Lofts, B. (1956), 'The effects of salinity changes on the respiratory rate of the prawn *Palaemonetes varians* (Leach)', *Journal of Experimental Biology* **33**, 730–736.
- Lowe, C. & Vance, V. (1955), 'Acclimation of the critical thermal maximum of the reptile *Urosaurus ornatus*', *Science* **122**, 73–74.
- Lowenstein, O. (1935), 'The respiratory rate of *Gammarus chevreuxi* in relation to differences in salinity', *The Journal of Experimental Biology* **12**, 217–221.
- Lutterschmidt, W. I. & Hutchison, V. H. (1997a), 'The critical thermal maximum: History and critique', *Canadian Journal of Zoology* **75**, 1561–1574.

- Lutterschmidt, W. I. & Hutchison, V. H. (1997b), 'The critical thermal maximum: Data to support the onset of spasms as the definitive end point', *Canadian Journal of Zoology* **75**, 1553–1560.
- MacArthur, R., Diamond, J. M. & Karr, J. R. (1972), 'Density compensation in island faunas', *Ecology* **53**, 330.
- MacArthur, R. & Wilson, E. (1967), *The theory of island biogeography*, Princeton University Press, Princeton.
- MacNeil, C., Dick, J. T. A. & Elwood, R. W. (2000), 'Differential physico-chemical tolerances of amphipod species revealed by field transplantations', *Oecologia* **124**, 1–7.
- MacNeil, C., Dick, J. T. & Elwood, R. W. (1997), 'The trophic ecology of freshwater *Gammarus* spp. (Crustacea: Amphipoda): Problems and perspectives concerning the functional feeding group concept', *Biological Reviews of the Cambridge Philosophical Society* **72**, 349–364.
- MacNeil, C., Prenter, J., Briffa, M., Fielding, N. J., Dick, J. T. A., Riddell, G. E., Hatcher, M. J. & Dunn, A. M. (2004), 'The replacement of a native freshwater amphipod by an invader: Roles for environmental degradation and intraguild predation', *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 1627–1635.
- Manush, S. M., Pal, A. K., Chatterjee, N., Das, T. & Mukherjee, S. C. (2004), 'Thermal tolerance and oxygen consumption of *Macrobrachium rosenbergii* acclimated to three temperatures', *Journal of Thermal Biology* **29**, 15–19.
- Maranhao, P., Bengala, N., Pardal, M. & Marques, J. C. (2001), 'The influence of environmental factors on the population dynamics, reproductive biology and productivity of *Echinogammarus marinus* Leach (Amphipoda, Gammaridae) in the Mondego estuary (Portugal)', *Acta Oecologica-International Journal of Ecology* **22**, 139–152.
- Maranhao, P. & Marques, J. C. (2003), 'The influence of temperature and salinity on the duration of embryonic development, fecundity and growth of the amphi-

- pod *Echinogammarus marinus* Leach (Gammaridae)', *Acta Oecologica - International Journal of Ecology* **24**, 5–13.
- Marques, J. C., Goncalves, S. C., Pardal, M. A., Chelazzi, L., Colombini, I., Fallaci, M., Bouslama, M. F., El Gtari, M., Charfi-Cheikhrouha, F. & Scapini, F. (2003), 'Comparison of *Talitrus saltator* (Amphipoda, Talitridae) biology, dynamics, and secondary production in Atlantic (Portugal) and Mediterranean (Italy and Tunisia) populations', *Estuarine Coastal and Shelf Science* **58**, 127–148.
- Marques, J. C. & Nogueira, A. (1991), 'Life cycle, dynamics and production of *Echinogammarus marinus* [Leach (Amphipoda)] in the Modego estuary (Portugal)', *Oceanologica Acta* **11**, 213–223.
- Marsden, I. D. (1973), 'Influence of salinity and temperature on survival and behavior of isopod *Sphaeroma rugicauda* from a salt-marsh habitat', *Marine Biology* **21**, 75–85.
- Mason, N. W. H., Lanoiselee, C., Mouillot, D., Wilson, J. B. & Argillier, C. (2008), 'Does niche overlap control relative abundance in French lacustrine fish communities? A new method incorporating functional traits', *Journal of Animal Ecology* **77**, 661–669.
- McCurdy, D. G., Forbes, M. R., Logan, S. P., Kopec, M. T. & Mautner, S. I. (2004), 'The functional significance of intersexes in the intertidal amphipod *Corophium volutator*', *Journal of Crustacean Biology* **24**, 261–265.
- McGill, B. (2003), 'Strong and weak tests of macroecological theory', *Oikos* **102**, 679–685.
- McKie, B. G., Cranston, P. S. & Pearson, R. G. (2004), 'Gondwanan mesotherms and cosmopolitan eurytherms: Effects of temperature on the development and survival of Australian Chironomidae (Diptera) from tropical and temperate populations', *Marine and Freshwater Research* **55**, 759–768.
- McLusky, D. S. (1967), 'Some effects of salinity on the survival, moulting and growth of *Corophium volutator* (Amphipoda)', *Journal of the Marine Biological Association of the United Kingdom* **47**, 607–617.

- McLusky, D. S. (1968), 'Some effects of salinity on the distribution and abundance of *Corophium volutator* in the Ythan Estuary', *Journal of the Marine Biological Association of the United Kingdom* **48**, 443–454.
- McLusky, D. S. (1969), 'The oxygen consumption of *Corophium volutator* relative to salinity', *Comparative Biochemistry and Physiology A - Molecular & Integrative Physiology* **29**, 743–753.
- McLusky, D. S. (1970), 'Salinity preference in *Corophium volutator*', *Journal of the Marine Biological Association of the United Kingdom* **50**, 747–752.
- McLusky, D. S., Bryant, V. & Campbell, R. (1986), 'The effects of temperature and salinity on the toxicity of heavy-metals to marine and estuarine invertebrates', *Oceanography and Marine Biology* **24**, 481–520.
- Meadows, P. S. (1964a), 'Experiments on substrate selection by *Corophium* species: Films and bacteria on sand particles', *Journal of Experimental Biology* **41**, 499–511.
- Meadows, P. S. (1964b), 'Experiments on substrate selection by *Corophium volutator* (Pallas): Depth selection and population density', *Journal of Experimental Biology* **41**, 677–687.
- Meadows, P. S. & Ruagh, A. A. (1981), 'Temperature preferences and activity of *Corophium volutator* (Pallas) in a new choice apparatus', *Sarsia* **66**, 67–72.
- MetOffice (2009), <http://www.metoffice.gov.uk/>.
- Meynard, C. N. & Quinn, J. F. (2007), 'Predicting species distributions: A critical comparison of the most common statistical models using artificial species', *Journal of Biogeography* **34**, 1455–1469.
- Möller, P. & Rosenberg, R. (1982), 'Production and abundance of the amphipod *Corophium volutator* on the West-coast of Sweden', *Netherlands Journal of Sea Research* **16**, 127–140.

- Moore, P. G. & Francis, C. H. (1986), 'Environmental tolerances of the beach-hopper *Orchestia gammarellus* (Pallas) (Crustacea, Amphipoda)', *Marine Environmental Research* **19**, 115–129.
- Morritt, D. & Spicer, J. I. (1996a), 'The culture of eggs and embryos of amphipod crustaceans: Implications for brood pouch physiology', *Journal of the Marine Biological Association of the United Kingdom* **76**, 361–376.
- Morritt, D. & Spicer, J. I. (1996b), 'Developmental ecophysiology of the beachflea *Orchestia gammarellus* (Pallas) (Crustacea: Amphipoda) .1. Female control of the embryonic environment', *Journal of Experimental Marine Biology and Ecology* **207**, 191–203.
- Morritt, D. & Spicer, J. I. (1996c), 'Developmental ecophysiology of the beachflea *Orchestia gammarellus* (Pallas) (Crustacea: Amphipoda) .2. Embryonic osmoregulation', *Journal of Experimental Marine Biology and Ecology* **207**, 205–216.
- Morritt, D. & Spicer, J. I. (1999), 'Developmental ecophysiology of the beachflea *Orchestia gammarellus* (Pallas) (Crustacea : Amphipoda : Talitridae) 3. Physiological competency as a possible explanation for timing of hatchling release', *Journal of Experimental Marine Biology and Ecology* **232**, 275–283.
- Muus, B. (1967), 'The fauna of Danish estuaries and lagoons. Distribution and ecology of dominating species in the shallow reaches of the mesohaline zone', *Medd Komm Dan-Fiskeri Javundersogelser Serie: Fiskeri* **5**, 1–316.
- Nagelkerken, I., van der Velde, G., Verberk, W. & Dorenbosch, M. (2006), 'Segregation along multiple resource axes in a tropical seagrass fish community', *Marine Ecology Progress Series* **308**, 79–89.
- Newell, R. (1979), *Biology of intertidal animals*, Third edn, Marine Ecological Surveys, Faversham, Kent.
- Normant, M. & Gibowicz, M. (2008), 'Salinity induced changes in haemolymph osmolality and total metabolic rate of the mud crab *Rhithropanopeus harrisii* Gould, 1841 from Baltic coastal waters', *Journal of Experimental Marine Biology and Ecology* **355**, 145–152.

- O'Connor, M. P., Kemp, S. J., Agosta, S. J., Hansen, F., Sieg, A. E., Wallace, B. P., McNair, J. N. & Dunham, A. E. (2007), 'Reconsidering the mechanistic basis of the metabolic theory of ecology', *Oikos* **116**, 1058–1072.
- Olden, J. D., Joy, M. K. & Death, R. G. (2006), 'Rediscovering the species in community-wide predictive modeling', *Ecological Applications* **16**, 1449–1460.
- Olivier, F. & Wotherspoon, S. J. (2006), 'Distribution and abundance of Wilson's storm petrels *Oceanites oceanicus* at two locations in East Antarctica: Testing habitat selection models', *Polar Biology* **29**, 878–892.
- Osovitz, C. J. & Hofmann, G. E. (2007), 'Marine macrophysiology: Studying physiological variation across large spatial scales in marine systems', *Comparative Biochemistry and Physiology A - Molecular & Integrative Physiology* **147**, 821–827.
- Ozinga, W. A., Bekker, R. M., Schaminee, J. H. J. & Van Groenendael, J. M. (2004), 'Dispersal potential in plant communities depends on environmental conditions', *Journal of Ecology* **92**, 767–777.
- Peer, D. L., Linkletter, L. E. & Hicklin, P. W. (1986), 'Life-history and reproductive-biology of *Corophium volutator* (Crustacea, Amphipoda) and the influence of shorebird predation on population-structure in Chignecto Bay, Bay of Fundy, Canada', *Netherlands Journal of Sea Research* **20**, 359–373.
- Potts, W. (1954), 'The energetics of osmotic regulation in brackish- and fresh-water animals', *Journal of Experimental Biology* **31**, 618–630.
- Prange, H. (1977), The scaling and mechanics of arthropod exoskeletons, in T. Pedley, ed., 'Scale effects in animal locomotion', Academic Press, London, pp. 169–181.
- Prinzing, A., Ozinga, W. A. & Durka, W. (2004), 'The relationship between global and regional distribution diminishes among phylogenetically basal species', *Evolution* **58**, 2622–2633.
- Pörtner, H. & Knust, R. (2007), 'Climate change affects marine fishes through the oxygen limitation of thermal tolerance', *Science* **315**, 95–97.

- Pörtner, H. O. (2002), 'Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals', *Comparative Biochemistry and Physiology A - Molecular & Integrative Physiology* **132**, 739–761.
- Pörtner, H. O., Lucassen, M. & Storch, D. (2005), Metabolic biochemistry: Its role in thermal tolerance and in the capacities of physiological and ecological function, in 'The Physiology of Polar Fishes', Vol. 22, Elsevier Academic Press, London.
- Rakusa-Suszczewski, S. & Lach, A. (1991), 'Respiration of *Orchomene plebs* (Hurley, 1965) and *Waldeckia obesa* (Chevreux, 1905) from Admiralty Bay (South Shetland Islands, Antarctic)', *Hydrobiologia* **223**, 177–180.
- Ray-Culp, M., Davis, M. & Stoner, A. W. (1999), 'Predation by xanthid crabs on early post-settlement gastropods: The role of prey size, prey density, and habitat complexity', *Journal of Experimental Marine Biology and Ecology* **240**, 303–321.
- Re, A. D., Diaz, F., Sierra, E., Rodriguez, J. & Perez, E. (2005), 'Effect of salinity and temperature on thermal tolerance of brown shrimp *Farfantepenaeus aztecus* (Ives) (Crustacea, Penaeidae)', *Journal of Thermal Biology* **30**, 618–622.
- Rice, A. N., Roberts, T. L. & Dorcas, M. E. (2006), 'Heating and cooling rates of eastern diamondback rattlesnakes, *Crotalus adamanteus*', *Journal of Thermal Biology* **31**, 501–505.
- Rodriguez, M. H., Ramirez, L. F. B. & Herrera, F. D. (1996), 'Critical thermal maximum of *Macrobrachium tenellum*', *Journal of Thermal Biology* **21**, 139–143.
- Rome, M. S., Young-Williams, A. C., Davis, G. R. & Hines, A. H. (2004), 'Linking temperature and salinity tolerance to winter mortality of Chesapeake Bay blue crabs (*Callinectes sapidus*)', *Journal of Experimental Marine Biology and Ecology* **319**, 129–145.
- Rundle, S. D., Bilton, D. T., Abbott, J. C. & Foggo, A. (2007), 'Range size in North American *Enallagma* damselflies correlates with wing size', *Freshwater Biology* **52**, 471–477.

- Sainte-Marie, B. (1991), 'A review of the reproductive bionomics of aquatic gammaridean amphipods - Variation of life-history traits with latitude, depth, salinity and superfamily', *Hydrobiologia* **223**, 189–227.
- Sander, F. & Moore, E. (1979), 'Temperature and salinity tolerance limits of marine gastropod *Murex pomum*', *Comparative Biochemistry and Physiology A - Molecular & Integrative Physiology* **64**, 285–289.
- Schmidt-Nielson, K. (1977), Problems of scaling: Locomotion and physiological correlates, in T. Pedley, ed., 'Scale effects in animal locomotion', Academic Press, London, pp. 1–21.
- Schmidt-Nielson, K. (2004), *Animal physiology: Adaptation and environment*, Fifth edn, Cambridge University Press, Cambridge.
- Schneider, S. D., Boates, J. S. & Forbes, M. (1994), 'Sex-ratios of *Corophium volutator* (Pallas) (Crustacea, Amphipoda) in Bay of Fundy populations', *Canadian Journal of Zoology* **72**, 1915–1921.
- Scholander, P., Flagg, W., Walters, V. & Irving, L. (1953), 'Climactic adaptation in Arctic and tropical poikilotherms', *Physiological Zoology* **26**, 67–92.
- Segerstråle, S. G. (1959), 'Synopsis of data on the crustaceans *Gammarus locusta*, *G. oceanicus*, *Pontoporeia affinis* and *Corophium volutator*', *Commentationes Biologicae (Soc. Sci. Fennica)* **20**, 1–23.
- Sepulveda, M., Vidal, M. A., Farina, J. M. & Sabat, P. (2008), 'Seasonal and geographic variation in thermal biology of the lizard *Microlophus atacamensis* (Squamata : Tropiduridae)', *Journal of Thermal Biology* **33**, 141–148.
- Sexton, E. (1924), 'The moulting and growth stages of *Gammarus*, with descriptions of the normals and intersexes of *G. chevreuxi*', *Journal of the Marine Biological Association of the United Kingdom* **13**, 340–401.
- Sexton, E. (1928), 'On rearing and breeding of *Gammarus* in laboratory conditions', *Journal of the Marine Biological Association of the United Kingdom* **15**, 33–55.

- Sexton, E. & Spooner, G. (1940), 'An account of *Marinogammarus* (Scheltenberg) gen. nov. (Amphipoda), with a description of a new species, *M. pirloti*', *Journal of the Marine Biological Association of the United Kingdom* **27**, 1–52.
- Smith, J. A., Millward, G. E. & Babbedge, N. H. (1995), 'Monitoring and management of water and sediment quality changes caused by a harbour impoundment scheme', *Environment International* **21**, 197–204.
- Sánchez-Moyano, J. E., García-Asencio, I. & García-Gómez, J. C. (2007), 'Effects of temporal variation of the seaweed *Caulerpa prolifera* cover on the associated crustacean community', *Marine Ecology-an Evolutionary Perspective* **28**, 324–337.
- Somero, G. N. (1997), Temperature relationships: from molecules to biogeography, in W. Dantzler, ed., '*Handbook of Physiology, Section 13: Comparative physiology*', Vol. 2, Oxford University Press, New York, pp. 1391–1444.
- Sorte, C. J. B. & Hofmann, G. E. (2005), 'Thermotolerance and heat-shock protein expression in Northeastern Pacific *Nucella* species with different biogeographical ranges', *Marine Biology* **146**, 985–993.
- Southwood, T. R. E. (1988), 'Tactics, strategies and templets', *Oikos* **52**, 3–18.
- Spicer, J. I. (1998), 'Is the reduced metabolism of hypogean amphipods solely a result of food limitation?', *Hydrobiologia* **377**, 201–204.
- Spicer, J. I. & Eriksson, S. P. (2003), 'Does the development of respiratory regulation always accompany the transition from pelagic larvae to benthic fossorial postlarvae in the Norway lobster *Nephrops norvegicus* (L.)?', *Journal of Experimental Marine Biology and Ecology* **295**, 219–243.
- Spicer, J. I. & Stromberg, J. O. (2003), 'Developmental changes in the responses of O₂ uptake and ventilation to acutely declining O₂ tensions in larval krill *Meganyctiphanes norvegica*', *Journal of Experimental Marine Biology and Ecology* **295**, 207–218.

- Statzner, B., Bonada, N. & Doledec, S. (2008), 'Predicting the abundance of European stream macroinvertebrates using biological attributes', *Oecologia* **156**, 65–73.
- Stillman, J. H. (2003), 'Acclimation capacity underlies susceptibility to climate change', *Science* **301**, 65–65.
- Stillman, J. H. & Somero, G. N. (2000), 'A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: Influences of latitude, vertical zonation, acclimation, and phylogeny', *Physiological and Biochemical Zoology* **73**, 200–208.
- Stock, J. (1967), 'Southernmost record of *Gammarus chevreuxi* Sexton, 1913', *Crustaceana* **12**, 200.
- Subida, M. D., Cunha, M. R. & Moreira, M. H. (2005), 'Life history, reproduction, and production of *Gammarus chevreuxi* (Amphipoda : Gammaridae) in the Ria de Aveiro, northwestern Portugal', *Journal of the North American Benthological Society* **24**, 82–100.
- Sutcliffe, D. W. (1984), 'Quatitative aspects of oxygen-uptake by *Gammarus* (Crustacea, Amphipoda) - A critical review', *Freshwater Biology* **14**, 443–489.
- Terblanche, J., Klok, C. J., Krafur, E. S. & Chown, S. L. (2006), 'Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (Diptera : Glossinidae): Implications for distribution modelling', *American Journal of Tropical Medicine and Hygiene* **75**, 186–186.
- Terblanche, J. S. & Chown, S. L. (2006), 'The relative contributions of developmental plasticity and adult acclimation to physiological variation in the tsetse fly, *Glossina pallidipes* (Diptera, Glossinidae)', *Journal of Experimental Biology* **209**, 1064–1073.
- Thrush, S. F. (1991), 'Spatial patterns in soft-bottom communities', *Trends in Ecology & Evolution* **6**, 75–79.
- Thrush, S. F., Halliday, J., Hewitt, J. E. & Lohrer, A. M. (2008), 'The effects of habitat loss, fragmentation, and community homogenization on resilience in estuaries', *Ecological Applications* **18**, 12–21.

- van der Meer, J. (2006), 'Metabolic theories in ecology', *Trends in Ecology & Evolution* **21**, 136–140.
- van Maren, M. J. (1975), 'The biology of *Cheato gammarus marinus* (Leach) and *Eulimnogammarus obtusatus* (Dahl), with some notes on other intertidal gammarid species (Crustacea, Amphipoda)', *Bijdragen tot de dierkunde* **45**, 205–223.
- Vidal, M., Ortiz, J. C. & Labra, A. (2002), 'Sexual and age differences in ecological variables of the lizard *Microlophus atacamensis* (Tropiduridae) from northern Chile', *Revista Chilena de Historia Natural* **75**, 283–292.
- Watkin, E. E. (1941), 'The yearly life cycle of the amphipod, *Corophium volutator*', *Journal of Animal Ecology* **10**, 77–93.
- Webb, T. J., Aleffi, I. F., Amouroux, J. M., Bachelet, G., Degraer, S., Dounas, C., Fleischer, D., Gremare, A., Herrmann, M., Hummel, H., Karakassis, I., Kedra, M., Kendall, M. A., Kotwicki, L., Labrune, C., Nevrova, E. L., Occhipinti-Ambrogi, A., Petrov, A., Revkov, N. K., Sarda, R., Simboursa, N., Speybroeck, J., Van Hoey, G., Vincx, M., Whomersley, P., Willems, W. & Wlodarska-Kowalczyk, M. (2009), 'Macroecology of the european soft sediment benthos: Insights from the MacroBen database', *Marine Ecology Progress Series* **382**, 287–296.
- Weibel, E. R. (1997), 'The future of physiology', *News in Physiological Sciences* **12**, 294–295.
- West, G. B. & Brown, J. H. (2004), 'Life's universal scaling laws', *Physics Today* **57**, 36–42.
- West, G. B., Savage, V. M., Gillooly, J., Enquist, B. J., Woodruff, W. H. & Brown, J. H. (2003), 'Why does metabolic rate scale with body size?', *Nature* **421**, 713–713.
- West, G. B., Woodruff, W. H. & Brown, J. H. (2002), 'Allometric scaling of metabolic rate from molecules and mitochondria to cells and mammals', *Proceedings of the National Academy of Sciences of the United States of America* **99**, 2473–2478.
- Wiesepape, L. M., Aldrich, D. V. & Strawn, K. (1972), 'Effects of temperature and salinity on thermal death in postlarval brown shrimp, *Penaeus aztecus*', *Physiological Zoology* **45**, 22–33.

- Wilkie, D. (1977), Metabolism and body size, in T. Pedley, ed., '*Scale effects in animal locomotion*', Academic Press, London, pp. 23–36.
- Wu, T. (1977), Introduction to the scaling of aquatic animal locomotion, in T. Pedley, ed., '*Scale effects in animal locomotion*', Academic Press, London, pp. 203–232.
- Ysebaert, T., Meire, P. & Buijs, J. (1993), 'The benthic macrofauna along the estuarine gradient of the Schelde estuary', *Netherlands Journal of Sea Research* **27**, 327–341.
- Yu, O. H. & Suh, H. L. (2002), 'Secondary production of *Synchelidium lenorostralum* (Amphipoda, Oedicerotidae) on a temperate sandy shore, southern Korea', *Journal of Crustacean Biology* **22**, 467–473.
- Yu, O. H., Suh, H. L. & Shirayama, Y. (2003), 'Feeding ecology of three amphipod species *Synchelidium lenorostralum*, *S. trioostegitum* and *Gitanopsis japonica* in the surf zone of a sandy shore', *Marine Ecology Progress Series* **258**, 189–199.
- Zweifel, R. (1957), 'Studies on the critical thermal maxima of salamanders', *Ecology* **38**, 64–69.